CANADIAN JOURNAL OF AGRICULTURAL SCIENCE

(*formerly* Scientific Agriculture)

VOLUME 36 JULY-AUGUST 1956 No. 4

AN EVALUATION OF OBJECTIVE METHODS FOR DETERMINING THE MATURITY OF CANNING PEAS[1]

W. E. Torfason, I. L. Nonnecke and G. Strachan

Canada Department of Agriculture, Lethbridge, Alberta

[Received for publication January 17, 1956]

ABSTRACT

A series of investigations was conducted during 1951, 1952 and 1953, to study objective methods for determining the maturity of canning peas. Both physical and chemical methods were studied, using three varieties of peas. Results indicated that all objective measurements of maturity were comparable to texture measured organoleptically. The three highest correlations of physical measurement of maturity were for the tenderometer, texturemeter and the pressure tester. Alcohol-insoluble solids and dry matter were the two chemical tests that gave highest correlations. Starch content varied from season to season, and from one variety to another, and thus was not a good indicator of maturity in peas. Dry matter content of peas was found to be correlated with maturity but the determination was not as reliable as alcohol-insoluble solids.

INTRODUCTION

Since the early years of food processing, when the prime consideration was preservation, increasing consumer preference for high quality food products has made the canning industry more quality-conscious. During the last 20 years, the development of better storage and transportation facilities for fresh foods, as well as the advent of the frozen food industry, has created a consumer choice for higher quality foods. As a result, canners are now endeavouring to harvest their crops at optimum maturity, i.e., the stage of development at which the flavour, texture, and nutritive value combine to give maximum quality.

Investigations involving many different methods of determining the maturity of vegetables before processing have been carried out at a number of institutions. The experiment reported in this paper was conducted at the Experimental Farm, Lethbridge, Alberta, to provide an evaluation of objective methods, both physical and chemical, for determining the maturity of peas grown in southern Alberta.

LITERATURE REVIEW

The quality of peas at maturity has been the subject of numerous articles, both technical and popular, during the past 20 years. Makower (9) emphasized that any objective test of maturity must, of necessity, be

[1] Contribution from the Horticulture Division, Experimental Farms Service.

[*The May-June number of this Journal (Volume 36, No. 3) was issued June 25, 1956*].

correlated with organoleptic appraisal by panels of human judges, since consumer taste preference is the ultimate test of quality.

The proportions of certain chemical constituents in plants change in relation to the stage of development. With this in mind, many workers have attempted to associate certain chemical properties with maturity of peas. The starch content of peas was found by Pollard *et al.* (11) to be one constituent which could be expected to increase with advancing maturity. Makower (9) reports only moderate increases in starch on the basis of variable results. It was considered that the proportions of the amylose and amylopectin fractions of the starch caused considerable error in the determinations. Therefore, starch content has not been considered a reliable measure of maturity.

The relationship of total and reducing sugars to maturity was not significant according to work reported by Makower (9). It was found that sugars seemed to be unstable before blanching and diffusible afterwards, and that there also appeared to be an increase followed by a decrease in sugars as peas mature.

Since starch analyses had not proved satisfactory, Kertesz (3) concluded that the substances insoluble in 80 per cent ethyl alcohol (starch, hemicelluloses, fibre and most proteins), would increase proportionally with maturity. His studies, and those of other investigators (4, 6, 7, 9, 11, 14, 15), have confirmed that alcohol-insoluble solids provide a reliable estimate of maturity as measured organoleptically.

The ascorbic acid content of peas was found to be associated with flavour but varied greatly with variety (6). This variation was so great that the determinations could not be correlated with maturity. Pollard *et al.* (11), on the other hand, observed that the ascorbic acid content of two varieties decreased slowly with increasing maturity.

Makower (9) reported that the refractive index, protein and lipid contents were not related to the maturity grade of peas. It was further noted that the quantity of expressed juice from raw peas was inadequate as a measure of maturity. However, Lynch and Mitchell (7) found that the volume of juice expressed at 500-lb. pressure correlated well with the alcohol-insoluble solids content.

The first attempt at physical quality grading involved the separation of peas into different size groups. This method provided rough maturity ranges for single varieties, but could not be considered reliable because size is dependent upon variety, location and other factors (9).

Specific gravity and brine flotation tests were found to be fairly satisfactory in establishing maturity grades of peas (9), but in more recent years have given way to other physical methods of determination.

The tenderometer, developed by Martin (10), was designed to measure the force required to shear and crush peas somewhat in the same manner as teeth in chewing. Good correlations between tenderometer readings and organoleptic appraisal and the alcohol-insoluble solids content were obtained by numerous workers (6, 7, 11, 12, 14, 15). These investigators felt that the tenderometer was a better measure of the maturity of raw than of processed peas. Some complaints regarding a suitable standard for adjusting the mechanism were noted.

The texturemeter, which measures the resistance to puncturing of a given volume of peas, was introduced by Christel (2). This instrument has been widely used although it has been reported to be less accurate than the tenderometer (10). A miniature tenderometer, similar in construction to the texturemeter but utilizing the shearing principle of the tenderometer, has been reported and has provided good results in limited tests (6). Other instruments which have been used in maturity determinations include the maturometer (7, 8, 12); the shearometer (7); the succulometer (7); the penetrometer (9), and the shear-press (5). Of these, the maturometer and the shearpress have provided the most satisfactory correlations with organoleptic appraisal, tenderometer readings, and alcohol-insoluble solids determinations. The literature indicates the reliability of alcohol-insoluble solids and of the "T" meters (tenderometer and texturemeter), and points to wider use of the maturometer and the shear-press in the evaluation of maturity in peas.

MATERIALS AND METHODS

Forty-pound pea samples were obtained at the receiving platform of a district canning plant in 1951, 1952 and 1953. Selection was based on the tenderometer readings of the ungraded samples in order to obtain a wide range of maturity. The samples were then transported as rapidly as possible to the laboratory where the peas were graded for size. Tenderometer, texturemeter, penetrometer and pressure tester (Chatillon corn tester) readings were recorded for each size grade. The dry matter, ascorbic acid and total sugar contents were determined on the raw samples. The remainder of each sample was processed for the determination of starch, alcohol-insoluble solids (A.I.S.) and for organoleptic appraisal. All methods of chemical analysis were those of the A.O.A.C. (1), with only minor modifications in some instances.

Triangular taste tests were used in 1951 and 1952 and the tasters who recognized duplicates registered their preferences. The resulting data were found to be unsuitable for statistical treatment. Therefore, in 1953, tasters were required to record a numerical score (1 to 10) where the lower scores indicated the more desirable textures.

Coefficients of correlation were calculated for all possible combinations of all determinations. Combined coefficients were also calculated where the Chi-square test (13) indicated that all samples were drawn from the same population.

RESULTS

In Table 1 the relationship of sieve size to maturity measurements is shown for three varieties, Wisconsin Early Sweet, Climax and Lincoln, as well as for the three varieties combined over the three-year period. It is evident from these measurements that considerable overlapping occurred from one sieve size to the next. This was true for all methods of measurement and for all varieties, although there was some variation in the degree of overlapping.

TABLE 1.—THE RELATIONSHIP OF SIEVE SIZE TO ALCOHOL-INSOLUBLE SOLIDS AND PHYSICAL MATURITY DETERMINATIONS, 1951-1953, INCLUSIVE

Variety	Sieve size	Tenderometer[1]		Texturemeter[1]		Per cent A.I.S.		Penetrometer[2]		Pressure tester[3]		Per cent dry matter	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mea
Wisconsin Early Sweet	5	113-164	137	105-145	129	12.4-16.5	14.4	2.7-4.3	3.6	153-205	173	23.6-25.9	24.4
	4	86-138	113	85-118	102	10.3-15.8	12.2	4.0-5.5	4.5	117-129	123	21.8-24.3	22.6
	3	80-111	92	61-92	78	7.4-11.8	9.3	5.6-7.0	6.1	72-113	91	19.9-22.7	20.7
	2	72-96	84	49-83	66	6.4-8.5	7.6	7.0-9.2	8.4	57-79	69	17.7-18.2	17.9
Climax	5	122-151	135	100-149	127	11.6-16.0	13.6	2.5-4.0	3.5	110-197	141	20.4-24.9	23.1
	4	100-137	113	80-121	102	10.2-13.9	11.3	3.9-5.9	4.7	90-140	107	20.9-22.9	21.5
	3	85-110	94	67-95	82	8.7-11.4	10.0	5.0-8.5	6.1	60-135	91	19.7-21.3	20.5
	2	70-78	75	75-80	78	—	—	6.4-6.9	6.7	50-105	84	20.0-21.1	20.5
Lincoln	5	92-126	113	90-122	107	10.2-14.4	13.0	3.2-5.5	4.1	95-139	108	20.3-24.0	22.7
	4	80-96	87	70-92	79	8.2-10.7	9.5	5.9-8.2	7.1	70-124	90	18.9-20.6	19.7
	3	71-90	80	60-80	70	7.2-8.9	8.1	7.6-8.2	7.9	45-96	72	17.8-19.2	18.5
	2	70-85	80	60-68	65	—	—	—	—	—	—	—	—
All three varieties	5	96-164	128	90-149	122	10.2-16.5	13.7	2.5-5.5	3.8	95-205	137	20.3-25.9	22.7
	4	80-158	104	70-121	94	8.2-15.8	11.0	3.9-8.2	5.3	70-140	107	18.9-24.3	21.3
	3	71-111	92	60-95	77	7.2-11.8	9.1	5.0-8.5	6.6	45-140	86	17.8-22.7	20.0
	2	70-96	80	49-83	70	—	—	6.4-9.2	7.3	50-105	78	17.7-21.1	19.5

Scale readings (mean of 3 determinations).
Depth of penetration in millimeters of 0.040-inch tapered needle (mean of 25 determinations).
Force (in grams) required to penetrate tissue with 0.065-inch rod (mean of 25 determinations).

TABLE 2.—THE COEFFICIENTS OF CORRELATION OF THE TASTERS' TEXTURE RATING WITH OTHER MATURITY MEASUREMENTS OF PEAS, 1953

Maturity measurement	N[1]	D.F.[2]	r
Tenderometer	29	20	0.8782**
Texturemeter	29	20	0.8690**
A.I.S.	28	19	0.8310**
Pressure tester	21	12	0.8937**
Dry matter	21	16	0.8620**
Total sugars	25	16	−0.4800*
Starch	25	16	0.5940**
Ascorbic acid	25	16	−0.5280*

[1] Number of comparisons.
[2] Degrees of freedom — ϵ (n−3), where n = number of comparisons per subsample.

* — P <0.05.
** — P <0.01.

The organoleptic tests made in 1951 and 1952 did not lend themselves to statistical treatment. However, they did indicate that the tasters preferred the more tender peas, as measured by the tenderometer, whether the differences were large or small. The numerical texture rating, used in 1953, bore this out, as may be observed by the coefficients of correlation appearing in Table 2.

All coefficients were significant at least to the 5 per cent level indicating that all objective measurements of maturity were comparable to texture measured organoleptically. The three highest coefficients were for the purely physical measurements (tenderometer, texturemeter and pressure tested) followed closely by alcohol-insoluble solids and dry matter content. The remaining measurements—total sugars, starch, and ascorbic acid—although associated with texture, were much less reliable than those previously mentioned.

TABLE 3.—COEFFICIENTS OF CORRELATION COMPARING PHYSICAL MEASUREMENTS OF MATURITY, 1951-1953, INCLUSIVE

	Tenderometer			Texturemeter			Penetrometer		
	N[1]	D.F.[2]	r	N[1]	D.F.[2]	r	N[1]	D.F.[2]	r
Texturemeter	102	75	0.9523**						
Penetrometer	50	32	−0.8481**	50	32	−0.9010**			
Pressure tester	47	32	0.8135**	46	31	0.8122**	20	14	−0.7830**

[1] Number of comparisons.
[2] Degree of freedom — ϵ (n−3), where n = number of comparisons per subsample.

** P <0.01.

TABLE 4.—COEFFICIENTS OF CORRELATION COMPARING PHYSICAL AND CHEMICAL MEASUREMENTS OF MATURITY, 1951-1953, INCLUSIVE

	Tenderometer			Texturemeter			Penetrometer			Pressure tester		
	N[1]	D.F.[2]	r	N	D.F.	r	N	D.F.	r	N	D.F.	r
A.I.S.	88	61	0.9731**	88	61	0.9533**	44	26	−0.9269**	39	24	0.7970**
Dry matter	54	36	0.9107**	54	36	0.8614**	25	16	−0.7010**	44	29	0.7065**
Ascorbic acid	77	53	−0.6620**	77	53	−0.6250**	38	23	0.7620**	44	29	−0.6015**
Total sugars	55	37	−0.5970**	55	37	−0.5710**	26	17	0.5460*	44	29	−0.2080

[2] Number of comparisons.
[1] Degrees of freedom ϵ (m − 3), where n = number of comparisons per subsample.
** P <0.05.
* P <0.01.

TABLE 5.—COEFFICIENTS OF CORRELATION COMPARING CHEMICAL MEASUREMENTS OF MATURITY, 1951-1953, INCLUSIVE

	A.I.S.			Dry matter			Starch			Ascorbic acid		
	N[1]	D.F.[2]	r	N	D.F.	r	N	D.F.	r	N	D.F.	r
Dry matter	47	29	0.9106**									
Starch	46	28	0.6140**	55	37	0.6000**						
Ascorbic acid	69	45	−0.6780**	57	39	−0.5975**	55	37	−0.3930**			
Total sugars	48	30	−0.6220**	57	39	−0.4800**	55	37	−0.4890**	57	39	0.3870*

[1] Number of comparisons.
[2] Degrees of freedom − ϵ (n − 3), where n = number of comparisons per subsample.
* P < 0.05.
** P < 0.01.

The relationship of the physical measurements to one another is illustrated by the correlation coefficients appearing in Table 3. Tenderometer and texturemeter readings exhibited a very high degree of association as is shown by the correlation coefficient of 0.9523. The relatively lower coefficients involving penetrometer and the pressure tester indicated greater variability in measurement as determined by these instruments.

A comparison of the association of all physical maturity measurements with each of the chemical measurements is shown in Table 4. Although the majority of correlation coefficients were significant, only two chemical determinations, alcohol-insoluble solids and dry matter content, could be considered closely related to the physical measurements. Starch content was found to be so variable that sub-sample groups could not be combined, total sugars provided comparatively low correlation coefficients, and ascorbic acid provided coefficients of intermediate values.

In comparing the chemical determinations with one another (see Table 5), only the correlation between alcohol-insoluble solids and dry matter was high. The comparison of alcohol-insoluble solids with the other determinations yielded much lower, though highly significant, coefficients. The remaining coefficients were high enough to show that the determinations compared were related, but that a great deal of variation existed.

DISCUSSION OF RESULTS

From the data presented, it would appear that size grading of peas was of value primarily in obtaining a processed product of uniform size but not necessarily of uniform maturity. The wide variation of maturity that occurs in single varieties of one size group would not permit satisfactory maturity grading on the basis of size. These observations are similar to those of Makower (9).

Tasters' Texture Rating correlated well with the physical measures of maturity, with dry matter and with alcohol-insoluble solids content. Indications are that objective measurements reliably estimate organoleptic texture. The other chemical determinations yielded much lower and less reliable correlations with the Tasters' Texture Rating, although all were significant.

The tenderometer and texturemeter provided sufficiently high correlations with one another and with alcohol-insoluble solids to permit interchangeable use of the instruments in estimating maturity. Kramer *et al.* (6) considered the texturemeter to be less precise than the tenderometer. This study indicates that the two instruments differed but little in precision and it is felt that the texturemeter could be used to advantage by fieldmen in assessing maturity prior to harvest. Its mobility and the need for only small samples make it an ideal instrument for such purposes. The results showed the penetrometer and pressure tester to be less precise than either of the "T" meters and, being tedious to operate, allow considerable human error to enter into the results.

The relationship of chemical determinations to physical measurements were similar in their relationship to the Tasters' Texture Rating, with one exception. Composite correlations for starch and each of the physical

measurements were not calculated because the Chi-square test indicated that sub-samples were not drawn from the same population. Starch content varied from season to season and from one variety to another, a condition which would explain the results of the Chi-square test. These results are contrary to those of Pollard *et al.* (11) but in agreement with those of other investigators (3, 7, 9). Dry matter content provided satisfactory correlation with organoleptic texture, with the "T" meters, and with alcohol-insoluble solids.

The remaining chemical determinations, ascorbic acid and total sugars were more variable providing relatively lower correlation coefficients, and it is felt they were not sufficiently reliable for optimum pea harvest predictions.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the contribution of: W. Westhora, Manager of the Cornwall Canning Company, Taber, Alberta, and his staff, for making samples available.

REFERENCES

1. Official and Tentative Methods of Analysis of the A.O.A.C. 6th ed. Association of Official Agricultural Chemists, Washington, D.C. 1945.
2. Christel, W. F. Texturemeter, a new device for measuring the texture of peas. The Canning Trade 60(10) : 34. 1938.
3. Kertesz, Z. I. The chemical determination of the quality of canned green peas. N.Y. Agr. Expt. Sta. Tech. Bull. 233. 1935.
4. Kramer, A. An objective testing of vegetable quality. Food Tech. 5 : 265-269. 1951.
5. Kramer, A., G. J. Burkhart, and H. B. Rogers, Jr. The shear-press, an instrument for measuring the quality of goods. Canner 112(5) : 34. 1951.
6. Kramer, A., L. E. Scott, R. B. Guyer, and L. E. Ide. Factors affecting the objective and organoleptic evaluation of quality in raw and canned peas. Food Tech. 4 : 142-150. 1950.
7. Lynch, L. J., and R. S. Mitchell. Physical measurement of quality in canning peas. C.S.I.R.O. Bull. 254. 1950.
8. Lynch, L. J., and R. S. Mitchell. The definition and prediction of the optimal harvest time of pea canning crops. C.S.I.R.O. Bull. 273. 1953.
9. Makower, R. U. Methods of measuring tenderness and maturity of processed peas. Food Tech. 4 : 403-408. 1950.
10. Martin, W. M. Apparatus for evaluating tenderness in peas. Canner 84(12) : 108. 1937.
11. Pollard, L. H., E. B. Wilcox, and H. P. Peterson. Maturity studies with canning peas. Utah Agr. Expt. Sta. Bull. 328. 1947.
12. Sayre, C. B. Comparison of the tenderometer and maturometer for measuring quality of raw peas. Proc. Amer. Soc. Hort. Sci. 63 : 371-377. 1954.
13. Snedecor, Geo. W. Statistical methods. 4th ed. Iowa College Press, Ames, Iowa. 1948.
14. Walls, E. P., and W. B. Kemp. Relationship between tenderometer readings and alcohol-insoluble solids in Alaska peas. Proc. Amer. Soc. Hort. Sci. 37 : 729-730. 1940.
15. Weckel, K. G., C. R. Rodriguez, D. C. Kuesel, H. Maze, and K. D. Demaree. Interrelationship between tenderometer, shear-press and alcohol-insoluble solids values of Alaska and Perfection peas. Food Packer 35(10) : 24-26. 1954.

STUDIES OF COLCHICINE-INDUCED TETRAPLOIDS OF TRIFOLIUM HYBRIDUM L.

I. CROSS AND SELF-FERTILITY AND CYTOLOGICAL OBSERVATIONS[1]

J. M. ARMSTRONG[2] AND R. W. ROBERTSON[3]

Canada Department of Agriculture, Ottawa

[Received for publication April 18, 1956]

ABSTRACT

Tetraploid alsike produced by colchicine treatment from diploid seed was initially high in forage yield but low in fertility. By selection of the more fertile plants in successive generations the fertility was raised to the diploid level by the seventh generation. Diploid and tetraploid plants were found to be incompatible, which should facilitate isolation and increase of tetraploid strains. A fairly high frequency of self-compatibility was noted upon selfing. This character was not retained in the progeny. Cytological studies of fifth generation plants showed a stable condition at meiosis. There was a high frequency of quadrivalents which separated regularly at anaphase. Cytological behaviour was not closely associated with varying fertility. The evidence supported the view that fertility is largely genetically controlled.

INTRODUCTION

In the last two decades, following the discovery of the use of colchicine for chromosome doubling by Dustan (5), plant breeders have utilized this method for obtaining new and improved varieties. In the production of autotetraploids in economic crop species partial sterility has been frequently encountered. Some success has been obtained in overcoming this sterility by the repeated selection of the more fertile plants in the generations following induced chromosome doubling. Some field crops in which the fertility of the tetraploids have been improved by selection are rye, barley, buckwheat, oil-seed rape, red and alsike clover.

Several varieties of tetraploid rye are now in commercial production. Tetra Petkus, a variety of German origin, is now licensed for sale in Canada and the acreage in this variety is steadily increasing. Muntzing (10) compared the derived tetraploids with their corresponding diploid varieties which have been produced in Sweden. While the tetraploids are not so fertile as the diploids, they have the compensating characters of higher kernel weight, superior seed-sprouting ability and better baking quality. There was some difficulty in raising the fertility of tetraploid rye by selection since Muntzing was unable to demonstrate, after growing isolates of tetraploid strains of steel rye in successive generations, that improved fertility had been obtained.

LITERATURE REVIEW

The extensive literature on autotetraploid barley has been well summarized by Smith (13). While the economic value of new tetraploids in barley has not been as striking as in the case of rye, a response toward increased fertility following selection has been reported. Ono (11) com-

[1] Contribution from the Forage Crops Division, Experimental Farms Service.
[2] Cytogeneticist.
[3] Assistant Cytogeneticist.

pared the fertility of 9 two-rowed and 9 six-rowed tetraploid lines with their diploid relatives. The two-rowed 4n lines averaged 73 per cent, and the 6-rowed 4n lines averaged 67 per cent of the 2n lines. As a result of selection in successive generations Ono obtained one strain 92 per cent as fertile as its corresponding diploid variety.

Sacharov *et al.* (12) produced several tetraploid strains of buckwheat. While there was the characteristic reduction in vigour in the first generation they were able to raise the fertility of the tetraploids to the diploid level after four generations of selection. In the tetraploids seed size, seed germination and frost resistance were superior to the diploids.

Armstrong (1) found that tetraploid strains of annual rape compared favourably with that of the diploids in yield. A larger seed size compensated for a slightly lower fertility.

Considerable work has been done in Scandinavian countries leading to the production of tetraploid strains of red clover and alsike. In both species there was an immediate increase in forage yield following chromosome doubling, but the fertility was greatly reduced. The improvement in fertility following the selection of the more fertile plants has been more marked with alsike than with red clover. For the 1945 season Frandsen (7) reported the seed yield of Otofte red clover as 262 kgm. per hectare with the corresponding tetraploid yielding 51 kgm. per hectare. With alsike, on the other hand, he reported that the seed yields of the more fertile tetraploid lines were equal to that of the diploids. Julen (8), in reviewing the work with clover in Sweden, stated that while excelling in forage yield the low fertility of the red clover tetraploids was still a drawback to their commercial use. The seed yield of the tetraploid strains of alsike was considered to be the equal of the diploids.

One might expect the lowered fertility of the autotetraploids would have a cytological basis. Darlington (3) considered it to be due to higher frequency of multivalent chromosome associations which frequently divide irregularly producing gametes with aneuploid chromosome numbers. From a study of 808 plants of tetraploid rye Muntzing (10) reported that 77 per cent of the plants had the normal chromosome number of 28; 13 per cent had 29, and 7 per cent had 27 chromosomes. More extreme deviates from the normal number were extremely rare. The observed level of aneuploidy could not fully account for the lowered fertility. From the studies of self-sterile and self-fertile lines of tetraploid maize Fischer (6) concluded that sterility was due mainly to genetic factors and only in part to cytological irregularities. Sparrow *et al.* (14), from a study of intravarietal and inter-varietal tetraploids in *Antirrhinum*, found that the varying fertility in the crosses was not correlated with the cytological behaviour. It is clear that the problem of lowered fertility in autotetraploids cannot as yet be completely explained.

In the present paper the results of a selection program with colchicine-induced tetraploids of alsike clover are detailed. The results of a cyto-logical study of a group of plants from the fifth generation are also reported.

PRODUCTION OF TETRAPLOIDS

Work leading to the production of tetraploid alsike at Ottawa was started in 1947. The diploid seed was from a commercial sample grown in

the alsike seed producing area of Prince George, B.C. The seedling method of colchicine treatment was followed. The seed was planted in flats and when the seedlings reached the single-leaf stage they were dug up, tied in small bunches and the tops were immersed in a 0.2 per cent aqueous solution of colchicine for 12 hours. They were then washed and replanted. As the plants came into bloom the pollen size was checked and 13 out of 175 plants were scored as tetraploids in whole or in part. One hundred seeds were secured by intercrossing the heads that appeared to be tetraploid. The plants grown from this seed were checked for chromosome numbers by root-tip smears. Fifty-six proved to be tetraploid and the remainder were diploid. No plants with aneuploid numbers were noted. Thirty-seven plants were selected for intercrossing. A total of 154 crosses were made, yielding 2500 seeds for an average of 16 seeds per head. Diploid check crosses made under the same conditions yielded 58 seeds per head.

Fertility of Tetraploids in Successive Generations

Populations of tetraploid alsike from the F_2 to F_7 generations have been grown and selection of the more fertile plants made in each generation. Data on fertility on an individual plant basis were recorded for all generations but the F_3, when the seed yield was considered on a line basis. Fertility was determined by threshing 5-10 heads per plant and averaging the result. Terminal heads on the main branches were chosen for sampling. Besides having the highest floret number, these heads were the best pollinated and matured well. Most of the generations were grown under field conditions but from the fifth generation forward supplementary use was made of a growth chamber where bench space was sufficient to grow 100 plants. At the onset of bloom a colony of honey bees was placed in the chamber to carry out pollination. In the first trial with the growth chamber 50 plants of tetraploid alsike and 50 of red clover were grown. The competition appeared to favour the red clover. On the latter, seed setting was uniform for all the heads on a plant and the seed yield was closely correlated with the seed yield of the same plants when they were grown in the field. With the alsike, on the other hand, the seed setting was variable on each plant and was considerably lower than comparable plants under field conditions.

TABLE 1.—FERTILITY OF TETRAPLOID ALSIKE IN SUCCESSIVE GENERATIONS

Generation	Where grown	No. of plants	Av. number of seeds per head
F_2	Field	515	20.4 ± 0.44
F_4	Field	298	32.0 ± 0.84
F_5	Field	55	64.1 ± 2.73
F_5	Growth chamber	50	37.3 ± 1.81
F_6	Growth chamber	95	78.9 ± 2.19
F_7	Growth chamber	86	107.9 ± 3.05
F_7	Field	127	95.4 ± 2.36

TABLE 2.—DISTRIBUTION OF F_2 AND F_4 GENERATIONS OF TETRAPLOID ALSIKE FOR FERTILITY

Generation	Median class values of seeds per head								Total
	4.5	14.5	24.5	34.5	44.5	54.5	64.5	74.5	
F_2 Actual	124	166	131	57	24	10	3	—	515
Per cent	24.0	32.3	25.5	11.0	4.7	2.0	0.6	—	100
F_4 Actual	20	46	70	67	46	30	14	4	297
Per cent	6.8	15.5	23.5	22.5	15.5	10.1	4.7	1.4	100

While the bees appeared to work the alsike bloom well there was perhaps a dilution with red clover pollen which lowered the effectiveness of the alsike pollen. In following tests in the growth chamber single species were grown and the results were much more consistent.

Table 1 summarizes the progress towards increased fertility in the successive generations. In two generations, fifth and seventh, fertility data on comparable lots of plants for field nursery and growth chamber were obtained. It may be seen from the table that the fertility has risen from 20 seeds per head in the second generation to approximately 100 seeds per head in the seventh. This level of fertility approximates that of diploid varieties. Table 2 shows the distribution of the plants for fertility in the F_2 and F_4 generations. In the F_2, there is a skewness to the distribution with the majority of the plants in the low fertility classes. In the F_4, the distribution approximates that of a normal curve. This distribution remains normal in later generations but with a shift of fertility classes. This has made it possible to raise the standard of selection for fertility in successive generations. In the F_2 population, plants setting 20 seeds or more per head were chosen, while by the F_7 generation only plants with a fertility in excess of 100 seeds per head were considered outstanding.

In the F_6 and F_7 generations some data were obtained in regard to head size in comparison to diploid plants. The heads were analysed for floret number and the fertility calculated on the basis of seeds per floret. The ovary of each pistil contains one to several ovules and under favourable conditions 2-3 seeds are produced. As shown in Table 3 the tetraploid contains about 80 florets per terminal head as compared with about 70 for the diploid. Considering the fertility in terms of seeds per head the tetraploid and diploid now rate about equal but when considered on a floret basis the diploid fertility is still slightly higher.

A number of heads with different grades of fertility were analysed on the basis of seeds per ovary. The results are shown in Table 4. When fertility is quite low, as in plant 2-61-5, a high proportion of florets are blank and the number of florets with two seeds is also low. As the fertility improves the ovaries with two and three seeds are proportionately higher. There are usually a number of unfertilized florets in even the best heads. This may be due to failure of bees to visit all the florets. Many ovaries

TABLE 3.—COMPARISON OF FERTILITY IN TERMS OF SEEDS PER FLORET

Generation	Where grown	No. of plants	Florets per head	Seeds per head	Seeds per floret
F_6	Growth chamber	94	77	80	1.04
F_7	Growth chamber	87	81	106	1.32
F_7	Field	122	79	95	1.20
Diploid	Field	24	72	103	1.43

TABLE 4.—SEEDS PER FLORET IN ALSIKE HEADS

Plant No.	Seeds per floret					No. of florets	No. of seeds	Seeds per floret
	0	1	2	3	4			
2-61-5	54	31	5			90	41	0.45
3-29-10	17	29	23	2		71	70	0.99
3-43-8	13	32	18	1		64	71	1.10
3-33-6	16	26	26	10		78	118	1.40
3-54-7		21	19	13	1	54	102	1.90
Diploid	9	26	32	3		70	99	1.40

show small rudimentary seeds, indicating an abortion of zygotes in an early stage or faulty endosperm development. It is also noted that, the higher the fertility of a plant or head, the better the sample in respect to plumpness of seed.

The data presented here indicate that, by selection, fertility in the tetraploid has been raised to the level of the average diploid plant where head fertility has been considered. Such characters as amount of bloom, resistance to shattering, uniformity in maturing, have not been measured. Adequate seed for plot trials on replicated basis will be necessary to obtain these data.

Self-Fertility

Most species of *Trifolium* are highly self-sterile, self- and cross-fertility being controlled by the oppositional allele system. In the derived tetraploids this system is more complex and operates to permit a higher level of self-fertility. Hypotheses for the behaviour of the oppositional allele system in tetraploids were first proposed by Lewis (9). These have been applied to species of *Trifolium* by Brewbaker and Atwood (2) who studied self-compatible tetraploid plants in white clover and alsike. They considered this self-compatibility was due to competitively interacting S alleles. They found that all plants in the selfed progenies of self-compatible plants are also self-compatible.

TABLE 5.—SELF-FERTILITY OF TETRAPLOID ALSIKE

Plant No.	Seeds per head									Total	Mean	Mean of parent
	0	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80			
10-5-4-10		5	3	6	3	1	3	2		23	29	54
7-3-3-3	2	7	2	0	2					13	11	16
7-5-2-2		2	1	2	1	1	1		1	9	32	26
6-2-4-7	1	3	6	2	1		2			15	20	50
10-5-1-1		7	4	2	2	3	2			20	23	40
10-5-1-6	4	3								7	2	21
11-4-1-7	9	3		1						13	3	24
11-4-1-8	3	3	2	1						9	8	27
7-3-3-1		3	3	2						8	14	18
15-4-2-7		8	3							11	8	25
15-4-2-8	8	6	3	1						18	6	31
16-1-5-3	5	9	3							17	5	44

A study was made of the self-fertility of our tetraploid alsike in later generations. A group of 62 F_6 plants were self-pollinated in the greenhouse using the usual toothpick technique. A check group of 42 diploid plants was also self-pollinated. The distribution of these two groups of plants in terms of seeds per head was as follows:

	Seeds per head						Total
	0-2	3-5	6-10	11-20	20-40	40-60	
Tetraploid	44	2	4	2	8	2	62
Diploid	40	1	1				42

Forty-four of the tetraploids were sterile or set one or two seeds per head. The self-fertility of 16, however, was so high that they might be considered self-compatible. Of the 42 diploid plants studied, 40 were self-sterile while two plants showed a slight self-fertility.

Small progenies of the more fertile tetraploid plants in this test were grown in the next generation. The self-fertility of these plants was determined and the results are given in Table 5. It will be noted the average fertility of each progeny is less than that of the parent plants. In the last seven progenies listed in the table this reduction in self-fertility is quite marked. These results differ from those obtained by Brewbaker and Atwood in that the self-fertility is not maintained in the progenies.

Fertility of Tetraploid × Diploid Cross

A frequent effect of induced tetraploidy is to impose a barrier of incompatibility between tetraploidy and diploidy. This is an advantage in isolating and increasing the tetraploid when it reaches economic status. To determine this in our material intercrossing tests were carried out between diploid and tetraploid plants. The results are shown in Table 6.

TABLE 6.—CROSS FERTILITY OF TETRAPLOID AND DIPLOID ALSIKE

Cross	No. of heads	No. of seeds	Seeds per head
2n × 2n	13	641	49.3
2n × 4n	6	2	0.3
4n × 2n	6	18	3.0
4n × 4n	29	530	18.3

The tetraploid plants were in the F_2 generation and the fertility of the tetraploid crosses is lower than that obtained in later generations. The diploid × tetraploid crosses were highly sterile only one head showing two seeds. In the reciprocal cross seeds were obtained from four of the six heads crossed for an average of three seeds per head. These were presumed

to be selfed seed since selfing tests showed a certain proportion of tetraploid plants to be self-compatible. It may be concluded that tetraploid alsike does not intercross with the diploid with any facility.

CYTOLOGICAL STUDIES

No cytological studies appear to have been carried out with *T. hybridum* beyond the determination of the somatic chromosome number. Efforts therefore were made to carry out studies to determine if meiotic behaviour in the tetraploids had a bearing on the fertility. The material consisted of 15 tetraploid plants in the F_5 generation, 4 tetraploid plants from the Swedish variety Tetra, and several diploid plants. The tetraploids were mostly pairs of sister plants and the fertility of the parent plants was known. Table 6 summarizes the meiotic data, the fertility of the parent plants and the pollen normalcy. Heads at the right stage for meiosis were fixed in 3 : 1 glacial-absolute and stored in a refrigerator. In making slides flower buds were dissected in a drop of aceto-carmine on the slide, the bud debris removed and the anthers smeared with a scapel. The cover-slip was then placed on and the slide heated over a steam bath for 30 seconds and then firmly pressed between blotting-paper. If microscopic examination showed useful stages the slide was made semi-permanent by ringing the slip with a mixture of certo, corn syrup and aceto carmine (1 : 1 : 1). Meiotic observations were made at first metaphase and first anaphase. In any given plant at least 10 cells were analysed. Additional observations were made of various pollen stages.

Table 6 lists the plants studied, the average chromosome association at first metaphase, and the range of number of quadrivalents. As a parameter of meiotic regularity the total percentage of associations as bivalents and quadrivalents has been calculated. These associations may be presumed to disjoin regularly at anaphase as compared to odd-numbered associations.

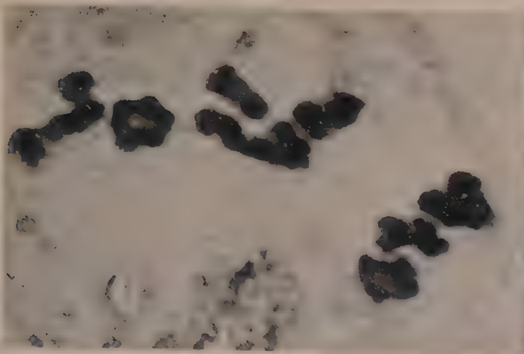
Cytologically all the tetraploids were essentially alike and have reached a reasonable state of stability. Applying the "t" test to the column of per cent bivalents and quadrivalents a difference of 1.05 per cent indicates significance. The average of 97.1 was exceeded by five plants and was less in the case of six plants. The Swedish tetraploid which is several generations in advance of our own was quite similar in its cytology.

One striking feature of the material was the high frequency of quadrivalents which averaged 4.8 for the Canadian material and 5.8 for the Swedish plants. Plate I, Figure 1, shows a first metaphase with 7 IV and 2 II. Plate I, Figure 2, shows 5 IV and 6 II. The most frequent range of IV's in a plant was from 4 to 7. The high average of quadrivalents indicates a relatively high frequency of chiasma formation at pachytene.

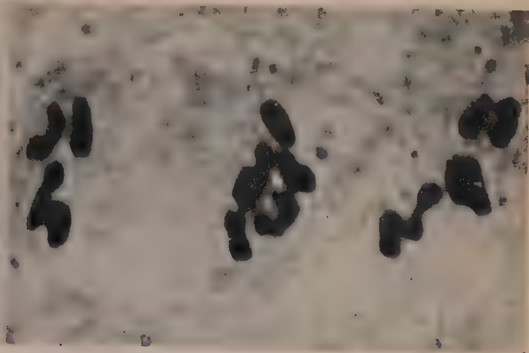
Frequently a high quadrivalent frequency is a cause of irregularity due to faulty disjunction at early anaphase. A careful study of this stage was made in several plants. Plate I, Figures 3 and 4, shows late metaphase stages when the chromosomes are in the process on disjoining. In Figure 3 there were apparently 5 IV and 6 II. The common mode of separation in quadrivalents is for adjacent chromosomes to go to opposite poles but

Plate I

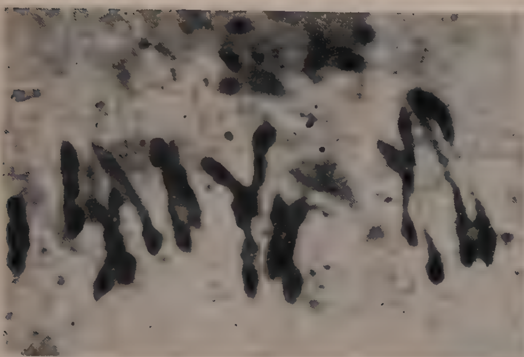
Meiosis in tetraploid alsike. These photomicrographs have been reproduced at a magnification of approximately × 2000.



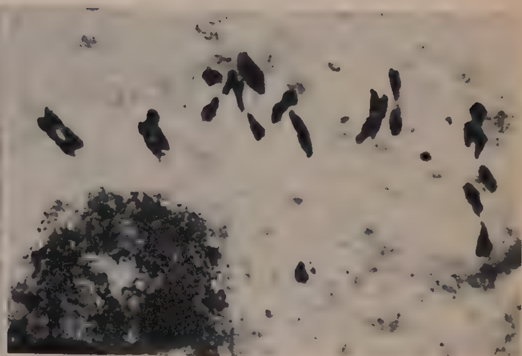
1



2



3



4

5

6

Figure 1. First metaphase showing 7 IV and 2 II.
Figure 2. First metaphase showing 6 IV and 4 II.
Figure 3. First metaphase with adjacent chromosomes in the quadrivalent oriented to the same pole.
Figure 4. Late metaphase showing types of dividing quadrivalents. Two univalents are present.
Figure 5. Early anaphase (one end of cell) in which chromosomes are still attached.
Figure 6. Late anaphase in which chromosome disjunction is complete.

in this material two adjacents go to the same pole. This is quite evident in the quadrivalent at the extreme right. Figure 4 shows this clearly in the quadrivalents to the left. At early anaphase, cells were observed in which separation into the 16 components was not complete. Figure 5 shows such an early anaphase (one end of the cell) in which there are 8 single chromsome units and 4 double units. This type of non-disjunction does not appear to a factor contributing to irregularity since all cells observed at late anaphase showed the separation into the 16 chromosomes as complete. Plate I, Figure 6, shows this separation very clearly.

The only source of irregularity appears to be the formation of univalents and trivalents. On the average such associations were low, being 0.37 per cell for univalents and 0.14 for trivalents. Only two type cells were observed of this kind. One type showed a trivalent and a univalent and the other showed two univalents. A cell containing a trivalent is certain to produce faulty gametes while in a cell containing two univalents one-half the gametic output would contain 15 to 17 chromsomes and the other half the normal number of 16. Of all cells examined in the 19 plants, 76.5 per cent showed no univalents, 14.0 per cent showed a univalent and a trivalent, and 9.5 per cent showed two univalents. Calculating one-half the latter as producing normal pollen, this would indicate 81.3 per cent of the cells should produce normal pollen.

Tetraploid pollen differs from normal diploid pollen in several respects. The measurement of several samples of 100 normal grains showed 27.1 $\pm$ 0.11 microns as the diameter of diploid pollen and 35.8 $\pm$ 0.21 microns as the diameter of tetraploid pollen. The difference is highly significant and in addition the attached errors show the tetraploid pollen to be somewhat more variable in size. The difference in pollen size is of considerable value in diagnosing induced tetraploids.

The last column in Table 6 gives the per cent of normal pollen in the various plants. The non-normal grains were very small or empty. The diploid checks showed 95.7 and 98.5 per cent normal pollen. The tetraploids averaged about 93 per cent normal pollen. There was no apparent correlation between pollen normalcy and meiotic regularity. There was also no relationship between the three characters given in the table, fertility of parent, meiotic behaviour and pollen normalcy.

DISCUSSION

As pointed out in the Literature Review, there are as yet no completely satisfactory explanations for the lowered fertility in autotetraploids or for the changes that occur when fertility is restored in later generations. The hypothesis first advanced by Darlington (3) that sterility in autotetraploids is due to the formation of multivalents which divide irregularly at anaphase producing gametes with aneuploid chromosome numbers does not seem to be borne out in subsequent research. The conclusion of Fischer (6), that sterility in autotetraploid maize is genetically controlled and is largely physiological in nature, appears to fit the general situation although this hypothesis is difficult to prove. The research with tetraploid alsike reported here does support the two points, that cytological behaviour is not closely associated with fertility and that the range of fertility present in a population from a given generation suggests genic control.

TABLE 7.—COMPARATIVE DATA ON DIPLOID AND TETRAPLOID ALSIKE FROM FIRST METAPHASE TO MATURE POLLEN

Plant No.	Type of plant	Fertility of parent	Chromosome associations					Range of IV	Per cent II and IV	Per cent normal pollen
			I	II	III	IV	VI			
1695-7	Diploid			8.0						95.7
1695-19	Diploid			8.0						98.5
21-2-9-4	Tetraploid	54.8	0.3	5.5	0.1	5.1		4-7	98.1	95.2
21-2-9-5	Tetraploid	54.8	0.4	2.6	0.4	6.2	0.2	4-7	93.8	91.6
21-4-2-2	Tetraploid	65.6	0.5	7.2	0.1	4.2		3-6	97.5	96.3
21-4-2-3	Tetraploid	65.6	0.2	5.2	0.2	4.8	0.2	2-7	92.5	90.4
21-6-3-2	Tetraploid	49.0	0.8	3.4	0.4	5.8		5-6	93.8	96.0
21-6-3-5	Tetraploid	49.0	0.8	4.8		5.4		4-7	97.5	89.6
21-10-10-1	Tetraploid	61.0		6.4		4.8		2-7	100.0	94.7
21-10-10-2	Tetraploid	61.0	0.5	5.1	0.3	5.1		4-7	95.6	93.1
22-5-3-5	Tetraploid	58.6	0.4	6.6		4.6		4-5	98.8	93.7
22-7-7-3	Tetraploid	76.7	0.2	6.0	0.2	4.8		4-6	97.5	94.6
22-7-7-1	Tetraploid	76.7	0.4	8.0		3.9		3-6	98.8	92.7
22-7-7-5	Tetraploid	76.7	0.3	5.8	0.1	4.8	0.1	4-5	96.3	96.2
23-7-6-3	Tetraploid	48.8		7.2		4.4		4-5	100.0	92.7
23-7-6-4	Tetraploid	48.8	0.2	8.4	0.2	3.6		3-4	97.5	85.2
21-24-5-3	Tetraploid	64.8	0.5	6.1	0.1	4.5		4-7	97.5	92.1
Av.			0.37	5.95	0.14	4.80			97.0	92.9
1695-20-1	Tetraploid		0.1	4.4	0.1	5.7		4-7	98.8	97.8
-2	Swedish		0.3	4.0	0.2	5.5	0.1	2-7	93.8	96.7
-3			0.6	3.4	0.2	6.0		5-7	96.3	97.8
-4				4.2		5.9		4-7	100.0	95.1

The parents of the F_5 tetraploids examined cytologically ranged in fertility from 50 to 75 seeds and the plants of the Tetra variety approximated 100 seeds per head. In spite of this range in fertility, the plants seemed to have the same meiotic pattern of high quadrivalent frequency at metaphase and regular anaphase disjunction. The frequency of trivalents and univalents which could be a source of aneuploid gametes was quite low and fairly uniform in distribution. Since all plants produced an abundance of normal appearing pollen it is unlikely that this could be a limiting factor. Sampling of plants for chromsome number in successive generations failed to show any aneuploidy as reported by Muntzing (10) in tetraploid rye. If such gametes occurred zygotes formed from their union apparently failed to function.

In the sampling of a given generation for fertility there was found to be a wide range in the distribution. Thus the F_2 ranged from 0 to 65 seeds per head and the F_7 from 55 to 175. This suggests that fertility is controlled genetically. The fact that fertility may be raised in the next generation by selecting the more fertile segregates would also support this view. Evidence for the operation of at least two genic systems affecting the complex character of fertility is present in the material.

Low fertility on the one hand appears to be due to factors that impose restrictions on fertilization. In alsike, with several ovules in each pistil, lack of fertilization would be shown by a reduction in the mean number of seeds per floret. An examination of flower heads with varying fertilities showed that this had occurred. If an ovary contains only one seed, pollination has been effected but the optimum fertilization of ovules has been reduced. In highly fertile heads two, three and sometimes four seeds are set per floret. The actual genetic basis for this situation may be inherent variation in the number of ovules per ovule as reported in white clover by Dessureaux (4), slow pollen tube growth or early abortion of young zygotes.

Another factor complex common to species of *Trifolium* which has a bearing on fertility is the oppositional factor allele system. This system is more complex in tetraploids than diploids and in certain tetraploid plants the factors may operate to permit self-compatibility. This is due to the interacting S alleles producing a condition in which the inhibiting stylar substance is ineffective in preventing pollen tube growth. While this condition should promote rather than decrease fertility it does suggest that a reverse situation might also occur in the tetraploids. There might be a lack of genic balance in the newly formed tetraploids which would upset the normal operation of this oppositional allele system. In later generations the adjustments made by selecting from the pool of modifying factors would make the reproductive processes operate more smoothly with a consequent improvement in the general level of fertility.

REFERENCES

1. Armstrong, J. M. A tetraploid form of annual rape induced by colchicine. Trans. Roy. Soc. Can. 44 : 21-38. 1950.
2. Brewbaker, J. L., and S. S. Atwood. Incompatibility alleles in polyploids. Proc. 6th Inter. Grassland Congress. State Coll. Pa. 1952.
3. Darlington, C. D. Recent advances in cytology. Blakiston Co., Philadelphia. 1937.

4. Dessureaux, W. Variation in the seed-setting ability of Ladino white clover. Sci. Agr. 30 : 109-57. 1950.
5. Dustan, A. P. Action de la colchicine sur le sarcome griffe Crocker, de la souris. Bull. Acad. Roy. Med. Belg. 14 : 487-8. 1937.
6. Fischer, H. E. An investigation of sterility in tetraploid maize. I. Pollen abortion II. Incompatibilities. Doctor's thesis, Cornell University, Ithaca, N.Y. 1939.
7. Frandsen, K. J. Iagttagelser over polyploid Former of Kulturplanter. Tidsskr. f. Planteavl. 51 : 640-65. 1948.
8. Julen, G. Clover and timothy breeding; Breeding of red clover and alsike clover. Hereditas Suppl. 44-45. 1949.
9. Lewis, D. Genetical studies in Pear IV. Pollen tube growth an incompatibility. J. Genetics 43 : 31-43. 1942.
10. Muntzing, A. Cytological properties and practical value of tetraploid rye. Hereditas 37 : 1-84. 1951.
11. Ono, T. Fertility of tetraploid barley. Jap. J. of Gen. 24 : 81-2. 1949.
12. Sacharov, V., S. L. Frolova, and V. Mansurova. High fertility of buckwheat tetraploids obtained by means of colchicine treatment. Nature 154 : 413. 1944.
13. Smith, L. Cytology and genetics of barley. Bot. Rev. 17 : 1-355. 1951.
14. Sparrow, A. H., M. L. Ruttle, and B. R. Nebel. Comparative cytology of sterile intra- and fertile intervarietal tetraploids of *Antirrhimum* majus L. Amer. J. Bot. 29 : 711-15. 1942.

ON THE CARBOHYDRATE COMPONENT IN LEAF EXTRACTS AND IN LEACHATES OBTAINED UNDER FOREST CANOPY[1]

K. D. MacLean[2] and W. A. DeLong[3]

[Received for publication February 22, 1956]

ABSTRACT

The carbohydrate contents of leaf extracts and of leachates obtained under deciduous forest canopy were estimated by the anthrone method. Variations during the growing season in the amounts of carbohydrate in the leachates were charted. It was established that rainfall passing through the canopy accumulated a carbohydrate component.

The amounts of carbohydrate removed in leachates obtained from leaves appeared, after the initial leaching, to increase when environmental conditions were favourable to microbiological activity.

The amounts of carbohydrate removed in leachates from the combined litter, A_0 layer and A_2 horizon of a sandy podzol appeared to be related more closely to the amount of precipitation during a rainfall than to conditions favouring microbiological activity. Leachates obtained from this source during June, 1953, had considerably higher iron saturation capacities, and much lower percentages of carbohydrate in the organic matter contained in them, than had leachates obtained from maple leaves on the same dates.

Evidence was obtained in support of the view that the major part of the carbohydrate component of leaf extracts is of relatively high molecular weight.

No consistent relationship was found between either the carbohydrate content or the furfural content and the iron saturation capacity values of leaf extracts.

INTRODUCTION

Previous reports from this laboratory (10, 11) have dealt with some general characteristics of leachates from decomposing leaves, and with the interaction of such leachates with soil-forming materials in the laboratory. The present communication deals primarily with attempts to estimate the amount and the nature of the carbohydrate component present in laboratory-prepared leaf extracts and in leachates obtained from leaves and other materials under a deciduous forest canopy. Comment also is made on the possible relationship of this component of extracts and leachates to their capacities to retain iron in solution or suspension.

EXPERIMENTAL MATERIALS AND METHODS

The procedure usually followed in the gathering of leaves in the series of investigations of which the present study forms a part, and the apparatus and technique employed in the collection of leachates, were described previously (3). However, the pine needles leached in 1952 and 1953 were stripped, in the spring of 1952, from small trees cut the previous autumn and stored over winter in an unheated building. The needles so obtained retained their green colour until placed on the tray. Humus layer (A_0) material was collected in an area where the litter consisted predominantly of white pine and eastern hemlock needles. The A-horizons were obtained by cutting out, from beneath the appropriate canopy, four sections, each measuring approximately 1′ x 3′ x 6″, to fill

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que. Macdonald College Journal Series No. 379.
[2] Assistant Chemist, Nova Scotia Agricultural College, Truro, N.S.
[3] Professor of Chemistry, Macdonald College, Que.

a tray. The objective was to transfer to the tray as much of the material above the B horizon and as little of the latter as possible. These sections were fitted in the tray snugly so that rainfall would pass through them rather than between or around them. Canopy drip solutions were collected by placing an empty tray under the appropriate canopy to catch rainfall and the water dripping from leaves and branches during rainfall. In each of the two seasons of the present investigation trays were freshly filled with the material to be leached during the first week of May. When leaves were to be leached 8.4 lb. was placed in a tray, except in case of the 1953 pine needles for which the amount was 16 lb. In 1952 approximately 6 cu. ft. of humus layer material were placed in the tray.

The solutions studied were derived from the source listed in Table 1. The soil series named in this table are those on which leaves were grown and from which humus and soil materials were obtained. The Rubicon, Uplands and Perrot series are sandy podzols, the Rubicon having characteristics of a ground-water podzol. The Grenville is a brown forest soil of clay loam texture.

Leaf extracts were prepared by shaking 10 gm. of air-dry leaf powder (<1.0 mm.) with 100 ml. of distilled water for one hour, filtering and making up to a volume of 140 ml. Iron saturation capacity estimations were made by the method described by DeLong and Schnitzer (4). Organic matter was estimated by the dichromate method (13), solutions first being evaporated to dryness on the water-bath.

TABLE 1.—NOMENCLATURE AND DESCRIPTION OF MATERIALS USED AS SOURCES OF EXTRACTS AND LEACHATES

Designation in text	*Description*	*Soil*
Beech	Leaves of Fagus grandifolia	Rubicon
Birch	Leaves of Betula populifolia	Uplands
Maple_G	Leaves of Acer saccharum	Grenville
Maple_P	*1952 sample;* a mixture of leaves of Acer saccharum and Acer rubrum	Perrot
	1953 sample; leaves of Acer saccharum	Perrot
Poplar	Leaves of Populus grandidentata	Rubicon
Pine	Needles of Pinus strobus	Rubicon
Beech–A	A_{00}, A_0 and A_2 taken from beneath a canopy of Fagus grandifolia	Rubicon
Pine–A	Same as preceding taken under a canopy of Pinus strobus	Rubicon
A_0–layer	A mixture of dead and decomposing material originating from pine and hemlock needles predominantly	Rubicon
Beech Canopy	Mainly beech (F. grandifolia) trees	Rubicon
Maple Canopy	Sugar maple (A. saccharum) trees	Grenville

Carbohydrate determinations were made by the method of Loewus (9) using a standard curve prepared from a standard invert sugar solution (12). No attempt was made to remove non-carbohydrate materials, such as tryptophane, giving colour with this reagent. Under the conditions of colour production and estimation used the full colour-producing effects of fructose and of pentoses would not be expected to be obtained. The data reported, therefore, are estimates of apparent carbohydrate content. These values are given in terms of grams of invert sugar equivalent. The anion exchange technique of Khym and Zill (8) was applied to the fractionation of the carbohydrate component of popular leaf extracts. The paper chromatographic procedure of Haugh *et al.* (6) was used in the tentative identification of the components of the fractions so obtained. The method of Adams and Castagne (1) was used for the estimation of furfural production from leaf extracts.

RESULTS AND DISCUSSION

The volumes of the maple$_G$ leachates collected in 1952 and 1953 with the amounts of apparent carbohydrate found in each are shown in Figure 1. The curves for carbohydrate removal show that, in both years, a maximum in the amount removed per leachate was reached late in June. The 1953 curve indicates extensive removal of carbohydrate in the first leachate of the season. Unfortunately the first two leachates collected in 1952 were not analysed for carbohydrate. The seasonal curves of carbohydrate elution for all species of leaves examined, and for the coniferous A_0-layer, showed a similar general trend to that exhibited by maple$_G$ leaves.

TABLE 2.—AMOUNTS OF CARBOHYDRATE ELUTED IN GRAMS OF INVERT SUGAR EQUIVALENT

Material leached	Carbohydrate eluted per season		Carbohydrate eluted—calculated to the annual leaf-fall weight	
	1952	1953	1952	1953
Maple$_P$	20.3	19.7	....	....
Maple$_G$	12.8	17.6	1.25	1.73
Poplar	6.7	10.7	0.33	0.53
Birch	9.5	....	0.47	....
Beech	4.6	....	0.29	....
Pine	4.6	3.2	....	....
A_0–layer	2.1	....		
Beech–A	1.1	1.0		
Pine–A	....	0.8		

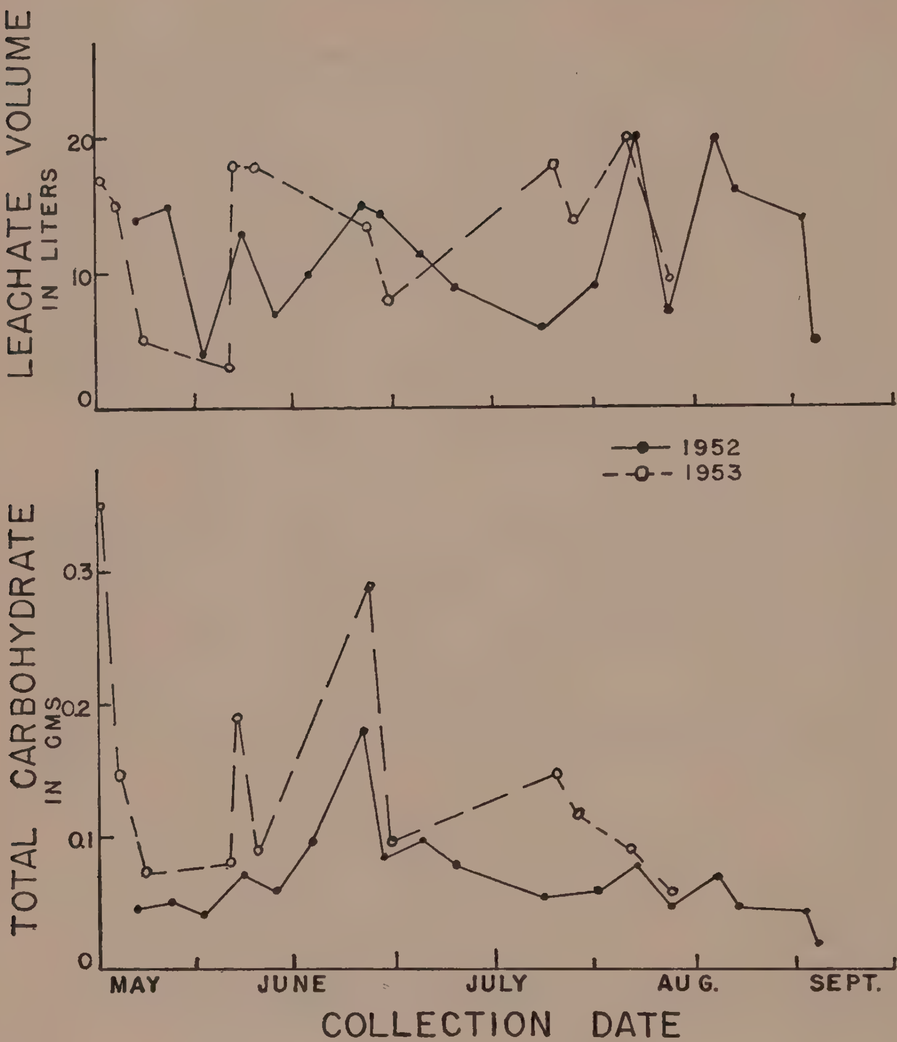


FIGURE 1. Volumes and carbohydrate contents of maple$_G$ leachates collected in 1952 and 1953

The volumes of leachate obtained from the beech A-horizons, and the apparent carbohydrate contents of these leachates are shown in Figure 2. Comparison of these data with those of Figure 1 shows clearly that the seasonal trend of carbohydrate elution is different from that obtained for leaves only, as shown for maple$_G$ leaves (Figure 1), and for the coniferous A_0-layer. Elution from the latter materials appeared to bear some relation to microbiological activity, a maximum being reached late in June and a minimum in late summer or early autumn. Moreover, there was, for leaves and for the A_0-layer, little apparent relation of carbohydrate removal to leachate volume. In contrast, the curves of Figure 2 suggest, for both seasons, a direct relation of volume of leachate to amount of

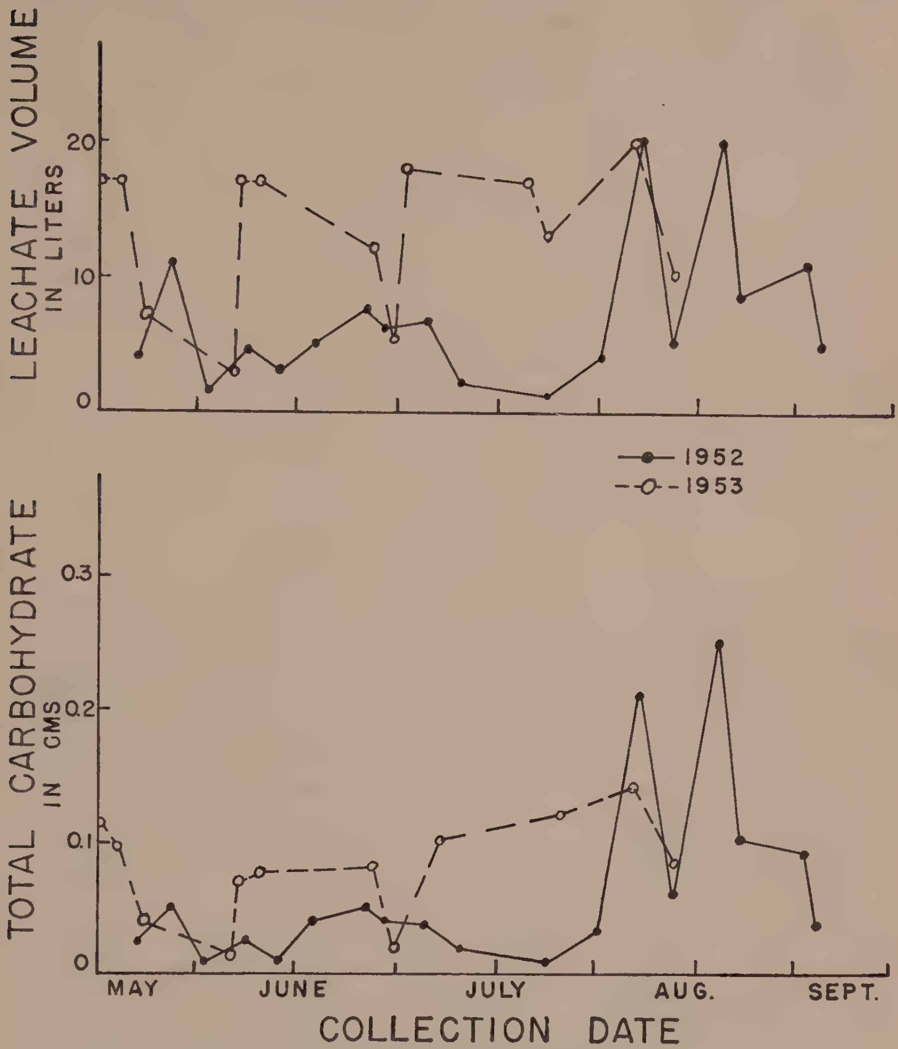


FIGURE 2. Volumes and carbohydrate contents of leachates from beech A-horizons in 1952 and 1953

carbohydrate eluted. The pine A-horizon studied in 1953 showed a similar correlation of leachate volume to carbohydrate elution.

The total amounts of carbohydrate removed in each of the two seasons, and these amounts calculated to the basis of the weight of the annual leaf-fall (3) in the case of the leaf leachates are presented in Table 2.

Since the weight of leaves of each deciduous species placed on a tray was the same, and since the weight of pine needles was approximately double that for the broad-leaved species, the data of Table 2 show that, per unit weight of material leached, maple leaves released much more carbohydrate than did leaves of the other species examined. Loss of carbohydrate from the A_0-layer was intermediate between that from leaves and that from A-horizons. Inspection of the values calculated to the

TABLE 3.—CARBOHYDRATE AS A PERCENTAGE OF THE ORGANIC MATTER IN LEACHATES COLLECTED UNDER FOREST CANOPY AND IN LEAF EXTRACTS PREPARED IN THE LABORATORY

Material	Leachates		Extracts
	1952	1953	
$Maple_P$	17.5	17.3	19.7
$Maple_G$	20.3	16.9	17.9
Popular	24.4	25.3	24.8
Birch	23.7	....	37.6
Beech	20.8	....	22.2
Pine	26.6	17.9	28.9
A_0–layer	15.9	....	....
Beech–A	7.7	4.8	....
Pine–A	....	4.8	....

basis of annual leaf-fall per 12 sq. ft. (the tray area) suggests that the actual amount of carbohydrate moving downward from the leaf litter is small, and that the amounts moving out of the A_0-layer and those calculated for removal of carbohydrate from the annual leaf-fall weights are of similar low order of magnitude. Possibly the relatively small elution of energy-yielding carbohydrate here observed is in part responsible for the rapid decrease in microbial numbers with depth in podzol profiles reported by Gray and McMaster (5).

The percentages of carbohydrate found in the organic matter of leachates and of leaf extracts are given in Table 3.

The data presented for leachates are the mean values for all collections of the 1953 season and for all but the first two leachates obtained in 1952. It may be significant that leaves of gray birch, poplar and white pine, species often occurring in association with podzolized soils, gave leachates the organic matter of which had a somewhat higher content of carbohydrate than was found for the organic matter of leachates from leaves of the other species examined. Probably of greater significance is the fact that the organic matter in the leachates from the A-horizon contains a much lower percentage of carbohydrate than do the other leachates. It appears that but little of this presumably respirable material (carbohydrate) reaches the B horizon, even in a sandy podzol.

The amounts of carbohydrate found in rainfall (canopy drip) solutions collected under maple and under beech trees are reported in Table 4.

This analysis of canopy drip solutions was suggested by the investigation of the mineral content of similar solutions by Ingham (7). The data of Table 4 show that rainfall passing through a deciduous forest canopy during the growing season may carry appreciable amounts of carbohydrate to the soil beneath. Comparison of the quantities of carbohydrate found in the leachates from $maple_G$ leaves with the amounts in

TABLE 4.—AMOUNTS OF CARBOHYDRATE* FOUND IN CANOPY DRIP SOLUTIONS

Collection date 1953	Maple canopy	Beech canopy
June 26	....	0.81
29	0.32	0.12
July 6	0.46	0.11
24	0.71	0.10
27	0.35	0.05
August 5	0.17	0.02
11	0.16	....
Totals	2.17	1.21

* In terms of grams of invert sugar equivalent.

the canopy drip solutions derived from the beech canopy under which the leaching trays were placed, in case of four rainfalls occurring during the interval June 29 to August 5, 1953, showed that the canopy contributed carbohydrate equivalent to 25 to 35 per cent of the amount found in the contemporaneous maple$_G$ leachates.

When the fractionation technique of Khym and Zill (8) was applied to poplar leaf extract, more than 60 per cent of the carboyhdrate passed through the column without being adsorbed. Elution distributed the adsorbed carbohydrate into three fractions removed in 0.005, 0.02 and 0.04 M tetraborate solutions respectively. The 0.005 M eluate contained 85 per cent of the total carbohydrate adsorbed by the column. The three eluate solutions were desalted by ion exchange resins and examined by paper chromatography (7), which indicated that fructose and glucose were present in the 0.02 and 0.04 M eluates respectively. The carbohydrate in the desalted 0.005 M eluate did not move from the origin when subjected to paper chromatography. This latter finding, together with the observation that most of the carbohydrate in the extract was not adsorbable on the column, indicated that the major part of the carbohydrate of poplar leaf extract was polymeric in nature. This conclusion was supported by the observation that, on clarification of this extract with lead acetate and sodium oxalate, the apparent carbohydrate content was reduced by 27.5 per cent; in the case of maple$_G$ extract, the reduction of apparent carbohydrate on such clarification amounted to 62.5 per cent.

The possibility that there might be a correlation between the amounts of carbohydrate in leachate solutions and the iron saturation capacities of these solutions was investigated. The results obtained are presented in Table 5.

Inspection of Table 5 shows that there is but little apparent correlation of the values being compared. In addition, it is seen that both characteristics of these leachates change from collection to collection,

TABLE 5.—COMPARISON OF CARBOHYDRATE CONTENTS OF LEACHATES WITH THEIR 50 PER CENT IRON SATURATION CAPACITIES*

Materials	Collected June 26, 1953		Collected June 29, 1953	
	Carbohydrate in leachate	50 per cent saturation capacity	Carbohydrate in leachate	50 per cent saturation capacity
$Maple_G$	266	96	157	83
$Maple_P$	258	109	154	79
Beech–A	53	199	33	230
Pine–A	54	230	32	203

* Both values expressed in terms of milligrams per gram of organic matter in the leachates; iron saturation capacities determined at pH 6.5 maintained with $Ca(OH)_2$ solution.

TABLE 6.—COMPARISON OF CARBOHYDRATE CONTENTS AND FURFURAL YIELDS OF LEAF EXTRACTS WITH THEIR 50 PER CENT IRON SATURATION CAPACITIES*

Materials	50 per cent saturation capacity	Carbohydrate content	Furfural content
Pine	83	421	14
Birch	133	377	33
Beech	148	222	16
$Maple_P$	175	197	8
Poplar	196	264	23
$Maple_G$	210	179	12

* All values given in terms of milligrams per gram of organic matter in the extracts; iron saturation capacities determined at pH 6.5 maintained with $Ca(OH)_2$ solution.

that variations in the ratio of carbohydrate to 50 per cent iron saturation capacity were in opposite directions for leachates from maple leaves and leachates from A-horizons, and that the much higher saturation capacity values of the leachates from the A-horizons were more constant than the corresponding values for leaf leachates.

The 50 per cent iron saturation capacities of leaf extracts were compared with the carbohydrate contents and the yields of furfural obtained from the extracts by the method of Adams and Castagne (1). The data are presented in Table 6.

Study of this table reveals very little correlation between carbohydrate content and saturation capacity, although there is some indication that when the carbohydrate content is high the saturation capacity tends to be low. Similarly, there is seen to be no evidence of a consistent relationship of the furfural content to the saturation capacity value.

REFERENCES

1. Adams, G. A., and A. E. Castagne. Some factors affecting the determination of furfural. Can. J. Res. 26, Sect. B : 314-324. 1948.
2. Barnett, A. J. G., and T. M. Miller. The determination of soluble carbohydrates in dried samples of grass silage by the anthrone method. J. Sci. Food Agr. 1 : 336-339. 1950.
3. Coldwell, B. B., and W. A. DeLong. Studies of the composition of deciduous forest tree leaves before and after partial decomposition. Sci. Agr. 30 : 456-466. 1950.
4. DeLong, W. A., and M. Schnitzer. Investigations on the mobilization and transport of iron in forested soils. I. The capacities of leaf leachates and extracts to react with iron. Soil Sci. Soc. Am. Proc. 19 : 360-363. 1955.
5. Gray, P. H. H., and N. B. McMaster. A microbiological study of podzol soil profiles. Can. J. Res. 8 : 375-389. 1933.
6. Haugh, L., J. K. W. Jones, and W. H. Wadman. Quantitative analyses of mixtures of sugars by the method of partition chromatography. J. Chem. Soc. 1702-1706. 1950.
7. Ingham, G. The mineral content of air and rain and its importance to agriculture. J. Agr. Sci. 40 : 55-61. 1950.
8. Khym, J. X., and L. P. Zill. The separation of sugars by iron exchange. J. Am. Chem. Soc. 74 : 2090-2094. 1952.
9. Loewus, F. A. The improvement in the anthrone method for the determination of carbohydrates. Anal. Chem. 24 : 219. 1952.
10. Lutwick, L. E., B. B. Coldwell, and W. A. DeLong. Leachates from decomposing leaves. I. Some general characteristics. Sci. Agr. 32 : 603-613. 1952.
11. Lutwick, L. E., and W. A. DeLong. Leachates from decomposing leaves. II. Interaction with soil-forming materials. Can. J. Agr. Sci. 34 : 203-213. 1954.
12. Methods of analysis, 7th ed. Assoc. of Official Agr. Chemists, Washington, D.C. 1950.
13. Peech, M., L. T. Alexander, L. A. Dean and J. F. Reed. Methods of soil analysis for soil fertility investigations. U.S.D.A. Circ. No. 757. 1947.

THE CONTROL OF VIOLET ROOT ROT IN ONTARIO[1]

N. J. Whitney[2]

Plant Pathology Laboratory, Canada Department of Agriculture, Harrow, Ontario

[Received for publication March 20, 1956]

ABSTRACT

Violet root rot of carrot was controlled in naturally infested muck in the greenhouse by the application of a 4% solution of formaldehyde at the rate of two litres per sq. yd. and by pentachloronitrobenzene (20%) applied at the rate of 50 gm. per sq. yd. In the field the disease was suppressed in decreasing order of effectiveness by the following chemicals: methyl bromide (Dowfume MC-2), formaldehyde, bleaching powder, and thiram (50%). One application of each of the first three materials gave significant disease reduction for two years. Other control measures include the use of varieties tolerant to the disease and harvesting the crop early, before the pathogen can become established.

INTRODUCTION

In the Thedford marsh in Ontario many carrot and celery crops have been severely affected by violet root rot, a disease caused by *Rhizoctonia crocorum* (Pers.) DC. (5, 6). Investigators in other countries (1, 2, 4) have shown that this disease can be controlled to some extent in clay or sandy soils by certain chemicals and cultural practices. None of these treatments appeared to be applicable to the control of the disease in the organic muck of the Thedford marsh. Therefore, after an initial investigation of the factors which influence disease development in this marsh (5), experiments were set up to find out if a practical control of violet root rot could be obtained on carrots in muck soil by the application of commonly used fungicides. In addition, a number of carrot varieties were tested for resistance to the disease. This paper reports the results of greenhouse and field experiments conducted concurrently in 1953 and 1954.

MATERIALS AND METHODS

All experiments were made on naturally infested muck. The field experiments were located on an infested area of a field in the Thedford marsh which had been under cultivation for 3 years. During that time, two crops of carrots and one crop of celery had been a total loss in an area approximately 500 ft. long and 40 ft. wide. Infested muck from this field was used for the greenhouse experiments.

In the greenhouse trials, chemical materials were thoroughly combined with the muck. The mixture, in 10-inch earthenware pots, was set aside for at least 2 weeks before the seed was planted. All treatments were randomized and replicated at least five times. In each pot carrot seed was planted at a depth of about one-half inch and the plants were thinned to four or five carrots. The muck was watered moderately, and after 16 weeks the plants were pulled and rated for disease incidence.

---

[1] Contribution No. 1525 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

[2] Associate Plant Pathologist.

Two separate field experiments were conducted to test the chemicals under natural conditions in the Thedford marsh. Randomized plots which measured 10 ft. by 6 ft. were used in all cases. In the first experiment, treatments were applied in the spring of 1953 and carried over the following year. In the second experiment, the treatments were applied in the spring of 1954 and tested for one season only. The chemicals, in dust or liquid form, were spread evenly over the muck and worked in to a depth of 4 to 6 inches. Methyl bromide gas (Dowfume MC-2) was applied under plastic sheets in the usual manner (3). Two weeks later carrot seed was planted in rows 14 inches apart in the seed bed which was prepared, planted, and cultivated by mechanized machinery used in the regular field operations. The carrots were harvested and examined for disease in mid-October of both years.

In a third field experiment ten varieties of carrots were tested for resistance to violet root rot.

Seed

Two varieties of carrot seed were used in the chemical experiments: Chantenay Red Cored in the greenhouse and Chantenay in the field experiments. Both varieties are commonly planted in the marsh and both were known to produce carrots of nearly the same degree of susceptibility to violet root rot. The following varieties were used in the variety test: Chantenay, Chantenay Red Cored, Chantenay Long Type, Oxheart, Hutchinson, Long Orange Improved, Imperator, Amsterdam, Morse's Bunching, and White Belgium.

Chemicals

Other investigators have cited results from the use of various chemical compounds in an attempt to control this disease. Of all the compounds tried bleaching powder reportedly gave the best control (1). Baudyš (2) stated that lime and formalin gave practically no control. In the present investigation the three chemical compounds referred to above, as well as the following additional ones, were tested: Arasan (thiram, 50% active material, non-wettable); aero-cyanamid (20.6% nitrogen, 70% hydrated lime); Brassicol (pentachloronitrobenzene 20%); methyl bromide (Dowfume MC-2), and ammonium nitrate.

Measurement of Disease

Disease incidence was determined at the end of each experiment and is expressed in terms of the percentage of carrots diseased for each treatment as well as the severity of the disease on the affected carrots. The disease severity, determined by the method outlined in an earlier publication (5), refers to the surface area which was diseased and is expressed as a percentage of the total surface area of the carrot.

GREENHOUSE EXPERIMENTS

In the first of two greenhouse experiments the following chemicals and amounts were added to naturally infested muck in 10-inch pots: thiram, 1 gm.; aero-cyanamid, 1.3 gm.; ammonium nitrate, 1.3 gm.; bleaching powder, two rates, 5.7 and 8.5 gm.; formaldehyde (4%), 100 cc.*. Each

* The equivalent amounts per square yard of muck are as follows: thiram, 20 gm.; aero-cyanamid, 26 gm.; ammonium nitrate, 26 gm.; bleaching powder at two rates, 4 oz., 6 oz.; formaldehyde, 2 litres.

TABLE 1.—THE INCIDENCE OF VIOLET ROOT ROT ON CARROTS GROWN IN THE GREENHOUSE IN NATURALLY INFESTED MUCK TREATED WITH VARIOUS CHEMICAL COMPOUNDS

Experiment I		Experiment II	
Treatment	Diseased carrots, per cent	Treatment	Diseased carrots, per cent
Check	100	Check	94.4
Thiram	100	Formaldehyde (4%)[2]	91.5
Aero-cyanamid	100	Brassicol	27.3
Ammonium nitrate	100	Formaldehyde (10%)[2]	13.0
Bleaching powder (4 oz.)	100	" (20%)[2]	0
Bleaching powder (6 oz.)	100	" (30%)[2]	0
Formaldehyde (4%)[1]	0	" (40%)[2]	0

[1] Two litres per sq. yd.
[2] One litre per sq. yd.

treatment plus the check (untreated) was replicated five times and twenty carrot seeds were planted in each pot. Counts of emerged plants and above-ground plant measurements were made to determine the effect of treatment on plant emergence and seedling vigour. Seed germination in all of the treated muck was better than in the untreated muck but analysis of variance of the data showed that these differences were not significant. After 6 weeks of growth none of the treatments had affected the carrot seedlings except the heavier application of bleaching powder which caused a significant stunting of the plants. Microscopic examination of the roots of these plants revealed no pathogenic fungi which might have accounted for this stunting. The plants in all of the pots were then thinned out and approximately four carrots were left in each pot to mature. When they were 16 weeks old the carrots were lifted and examined for violet root rot.

Examination of Table 1 shows that the only chemical which effectively reduced the disease was formaldehyde. All the carrots in this treatment were healthy and perfectly free from disease (Figure 1). None of the other treatments reduced the disease below that in the check.

In the second greenhouse experiment, 35 pots of naturally infested muck were treated in groups of five as follows: check (no treatment); pentachloronitrobenzene (20%), 2.5 gm. per pot; five different strengths of formaldehyde (4, 10, 20, 30, and 40%), each applied at the rate of one litre per square yard (50 cc. per pot). Carrot seed was planted and, in each pot, approximately five seedlings were left to grow to maturity. Sixteen weeks later the mature carrots were examined for violet root rot.

In Table 1, Experiment 2, all treatments are seen to have reduced the disease; the results were significant at the 1% level. Formaldehyde

FIGURE 1. The effect of formaldehyde on violet root rot of carrot in the greenhouse: Healthy carrots grown in infested muck treated with a 4% solution of formaldehyde at the rate of 2 litres per square yard (*left*), and diseased carrots grown in untreated muck (*right*).

FIGURE 2. The effect of methyl bromide on violet root rot of carrot in the field: Carrots grown in muck treated with methyl bromide (*left*), and diseased carrots grown in untreated muck (*right*).

(4%) at the rate of only 1 litre per square yard did not control the disease as well as the heavier application of 2 litres per sq. yd, shown in the first experiment. Pentachloronitrobenzene and formaldehyde (10%) reduced the disease considerably, while the three stronger solutions of formaldehyde completely eliminated it.

These two experiments show that under greenhouse conditions violet root rot of carrot can be prevented by formaldehyde and controlled by pentachloronitrobenzene.

FIELD EXPERIMENTS

Chemical Amendments

In May, 1953, experimental plots were set up in naturally infested muck in the Thedford marsh. Four treatments, replicated three times, were applied at the following rates per square yard: formaldehyde (4% solution), 1 litre; lime, 13.3 oz.; bleaching powder, 2 oz.; methyl bromide, 78 ml.; check (no treatment). Two weeks later Chantenay carrot seed was planted in each plot and the carrots were harvested and examined for disease on October 15. In May, 1954, these plots were again planted with Chantenay carrot seed to determine the duration of effectiveness of the treatments. The carrots were examined for disease in October, 1954. The results of this experiment are shown in Table 2.

Table 2 shows that more disease developed in the check plots in 1953 than in 1954. However, in both of the years each treatment significantly reduced the disease, and this reduction indicates the effectiveness of one application for two years.

In May, 1954, new plots were set up adjacent to the old ones. Each unit of six plots was replicated six times; a check plot received no treatment but the remaining five plots in each unit were treated with chemicals at the following rates per square yard: thiram, 18 gm.; aero-cyanamid, 26 gm.; formaldehyde (4% solution), 1 litre; formaldehyde (10 % solution),

TABLE 2.—INCIDENCE OF VIOLET ROOT ROT ON CARROTS AFTER THE APPLICATION OF CHEMICAL TREATMENTS TO NATURALLY INFESTED MUCK IN 1953

Treatment	Diseased carrots, per cent		Disease severity, total surface diseased[1], per cent	
	1953 crop	1954 crop	1953 crop	1954 crop
Check	76.8	59.5	40.8	45.8
Bleaching powder	35.9**	52.8*	28.6**	38.1*
Lime	52.0**	33.0**	26.8**	30.3**
Formaldehyde (4%)	38.0**	25.2**	26.8**	29.2**
Methyl bromide	5.7**	13.7**	15.1**	15.8**

[1] For diseased carrots only.
* Significant at the 5% level.
** Significant at the 1% level.

TABLE 3.—THE EFFECT OF CHEMICAL TREATMENT ON THE INCIDENCE OF DISEASED CARROTS IN NATURALLY INFESTED MUCK

Treatment		Diseased carrots, per cent	Disease severity, total surface diseased[1], per cent
Check		68.2	58.9
Aero-cyanamid		84.2	71.1
Formaldehyde (4%)		64.2	56.5
Thiram		53.7	65.6
Formaldehyde (10%)		42.7	49.7
Methyl bromide		11.7	24.3
L.S.D.	0.01	4.6	25.6
	0.05	3.5	18.9

[1]For diseased carrots only.

1 litre; methyl bromide, 39 ml. Two weeks later Chantenay carrot seed was planted in each plot. Root examinations were made in October and the results are shown in Table 3.

All treatments except aero-cyanamid significantly reduced the percentage of carrots diseased, but only methyl bromide significantly reduced the severity of the disease (see also Figure 2).

These two experiments show that in the field violet root rot can be significantly reduced by methyl bromide and formaldehyde and controlled to a lesser extent by bleaching powder, lime, and thiram.

Variety Test

In May, 1954, ten varieties of carrot seed were planted in naturally infested muck to be tested for resistance to violet root rot. In each plot there were two rows 12 ft. long and each unit of ten plots was replicated six times. The plots were completely randomized within each unit. The carrots were examined for disease in October.

As Table 4 shows, the varieties Chantenay and Chantenay Red Cored were the least susceptible. Although there was no significant difference between the number of carrots diseased in these two varieties, the disease was significantly less severe on Chantenay.

DISCUSSION AND CONCLUSIONS

Since *R. crocorum* is indigenous in the Thedford marsh (5) violet root rot is encountered and must be combated as soon as the land is broken up for the production of the first crop. In the experiments reported in this paper the growing of tolerant varieties and the chemical treatment of the muck with a suitable fungicide have been shown to provide effective con-

TABLE 4.—INCIDENCE AND SEVERITY OF VIOLET ROOT ROT ON TEN VARIETIES OF CARROTS GROWN IN NATURALLY INFESTED MUCK

Variety		Mean number of carrots diseased (in degrees)[1]	Disease severity, total surface diseased[2], per cent
Chantenay Long Type		64.14	69.5
Oxheart		59.27	49.8
Morse's Bunching		59.30	69.0
Imperator		52.64	70.1
Hutchinson		49.52	45.8
Long Orange Improved		47.22	49.8
Amsterdam		44.33	57.3
White Belgium		43.72	46.3
Chantenay Red Cored		36.20	51.3
Chantenay		35.06	30.5
L.S.D.	0.01	17.99	1.9
	0.05	15.23	1.5

[1] Analysis of the percentage of carrots diseased was done by means of an angular transformation.
[2] For diseased carrots only.

trol of the disease. In the case of carrots, it is evident from the results of the variety test that the most tolerant varieties are Chantenay and Chantenay Red Cored, both of which are acceptable for bunching, canning, and winter storage. The variety Nantes, an early bunching carrot often grown in the Thedford marsh for table use, was not included in the test because of its extreme susceptibility to violet root rot as observed in a preliminary experiment.

Some of the chemicals used in these tests controlled the disease while others did not. In the field, methyl bromide gave the best control but this chemical fumigant depressed the stand of carrots considerably. In both of the field experiments the reduction in the number of diseased carrots shows that most of the treatments reduced the amount of inoculum in the muck. When the inoculum was greatly reduced, as was the case with methyl bromide, there was also a considerable reduction in the severity of the disease. This appears to be related to a mass action effect in which the severity of the disease depends on the amount of inoculum present initially and not on the subsequent development and spread of the disease following only a few infection points on the carrot. This observation lends further support to the mass action effect as it applies to this disease and which was cited in the discussion in an earlier publication (5). Brassicol was effective in the greenhouse but was not tested in the field because

it was in limited supply. A curious phenomenon occurred with the use of bleaching powder. In the field the disease was reduced by an application of 2 ounces of bleaching powder per square yard. In the greenhouse, on the other hand, heavier applications of 4 to 6 ounces per square yard had no effect on the disease. This difference in fungicidal activity may have been due to temperature differences in the two experiments. At the time when the chemical was applied in the field, the temperature of the muck at a depth of 4 inches was approximately 50°F., while the temperature of the muck in the greenhouse when the chemical was applied was 70°F. At the higher temperature in the greenhouse the chlorine probably diffused out of the soil much faster than at the lower temperature in the field and, since *R. crocorum* forms sclerotia in the muck, the rapid dissipation of the killing agent would not have much effect on this fungus.

In the field experiments the formaldehyde solutions were applied at the rate of 1 litre per square yard of muck to determine if this chemical would give reasonable control at this low rate. The results of the trials are similar to those obtained in the greenhouse when the solutions were applied at the same rate. A significant reduction occurred in the one set of plots (Table 2) but not in the other (Table 3), which shows that the fungicidal action of formaldehyde (4%) at 1 litre per square yard is somewhat unpredictable and may be influenced by weather conditions. Considerably more rain fell in 1954 than in 1953 which might have accounted for the reduced effectiveness of the 1954 treatment. Presumably, heavier applications in the field would have reduced the disease further. Such a reduction occurred in the greenhouse when formaldehyde was applied at the rate of 2 litres per square yard.

Since formaldehyde gave positive results in all of the tests and did not inhibit growth of the carrots, it may be recommended as an effective fungicide for the control of violet root rot of carrot, at the rate of 2 litres of a 4% solution per square yard. Usually violet root rot occurs in the field in patches which can be marked off at harvest time and conveniently treated with formaldehyde before the next crop is planted. Such a treatment over a period of 2 or 3 years should effectively reduce the amount of inoculum in the muck at reasonable cost and with comparatively little labour.

In an earlier publication on violet root rot of carrot (5) it was pointed out that in fields where both early and late carrots are grown*, only the late carrots are affected by the disease. This late attack was related to the age of the carrot, and it was proved that the carrots were not attacked before they were 8 weeks old and that from this time until harvest both incidence and severity of the disease increased. In general, this has been the pattern of disease development in the Thedford marsh in both carrots and celery from 1947 to 1954 during which period a large acreage of marsh land was brought under cultivation. The early crops in this area have always been free from violet root rot. Therefore, to circumvent this disease, early cropping became expedient. This, combined with the other

* The early and late carrots are both sown at the same time in the spring. Those referred to as early are pulled in August for bunching while the late ones are left in the field until October.

control measures cited above, will make it possible to keep many acres of the highly desirable muck land of the Thedford marsh in profitable production.

ACKNOWLEDGEMENT

The author wishes to express his appreciation to L. W. Koch for helpful suggestions during the preparation of this manuscript.

REFERENCES

1. Barker, B. T. P., and C. T. Gimingham. Further experiments on the Rhizoctonia disease of Asparagus. Univ. of Bristol. Ann. Rept. and Hort. Res. Sta. 1917 : 28-34. 1917.
2. Baudyš, E. Studium houby *Rhizoctonia violacea*. Bull. Czecksolov. Acad. Agr. V, 8 : 693-697. 1929. Abstr. in Rev. Appl. Mycol. 9 : 219. 1930.
3. Dieter, C. E., and L. L. Coulter. Dowfume MC-2. . . . For fumigation of plant beds. Down to Earth 4 (4) : 1-5. 1949.
4. Hull, R. Sugar beet diseases. (Gt. Brit.) Ministry Agr. and Fisheries. Bull. 142. H.M. Stationery Office, London. 1950.
5. Whitney, N. J. Investigations of *Rhizoctonia crocorum* (Pers.) DC. in relation to the violet root rot of carrot. Can. J. Botany 32 : 679-704. 1954.
6. Whitney, N. J. Violet root rot of celery. Plant Disease Rept. 38 : 462-463. 1954.

GENOME ANALYSIS OF *AEGILOPS JUVENALIS*[1]

By R. C. McGinnis[2]

Canada Department of Agriculture, Winnipeg, Manitoba

[Received for publication May 24, 1956]

ABSTRACT

A number of interspecific and intergeneric F_1 hybrids involving species of *Triticum* and *Aegilops* were studied cytologically at metaphase I of meiosis to determine the genomes present in the hexaploid *Ae. juvenalis* (Thell.) Eig. The amount and kind of pairing at meiosis was employed in establishing genome relationships. It was concluded that *Ae. juvenalis* has the D genome of *Ae. squarrosa* L. somewhat altered from the D of *T. vulgare* Vill. In addition there was evidence that the C^u genome of *Ae. umbellulata* Zhuk. is also present in *Ae. juvenalis.* The third genome was not determined.

INTRODUCTION

Because of the economic importance of the genus *Triticum*, the other members of the sub-tribe Triticinae—*Aegilops*, *Agropyron*, *Secale* and *Haynaldia*—have long proven to be of interest from a cytological, genetical and phylogenetical standpoint. These genera all have a basic chromosome number of 7 and it is generally agreed that early in their evolution these genera were derived from a common ancestor. Even today a high degree of fertility exists between species and genera indicating a marked amount of residual genic similarity; in fact complete genomes have been shown to be common to several species.

Within the genus *Aegilops* considerable work has been done to determine genome relationships of different species. These relationships are based primarily on the amount and kind of pairing at metaphase I of meiosis in interspecific and intergeneric F_1 hybrids. The present study deals mainly with the hexaploid species *Ae. juvenalis* (Thell.) Eig for which no cytological evidence of its genome constitution has yet been presented.

REVIEW OF LITERATURE

Kihara (9) in extensive studies of the *Aegilops* proposed that five genomes are present in this genus, namely, C, D, J, M and S. However, in his recent revision (10) only four genomes are included, the J having been found to belong to the M group. Within the C, M and S genomes evolutionary modifications have occurred resulting in the formation of new species. Such species now have only partial homology with the original species. In order to signify these differences, a superscript has been added to the basic genome symbol, e.g. C^u for the genome of *Ae. umbellulata* Zhuk. to indicate that it has diverged from the C genome of *Ae. caudata* L.

Taxonomically, *Ae. juvenalis* was recognized by Eig (3) as belonging to the same section, Pachystachys, as *Ae. Squarrosa* L., a diploid species now known to have contributed the D genome to *Triticum vulgare* Vill.

[1] Contribution No. 190, Cereal Crops Division, Experimental Farms Service, Ottawa. Part of a thesis submitted to the University of Manitoba in partial fulfilment of requirements for degree of Doctor of Philosophy.
[2] Cytologist, Cereal Breeding Laboratory, Winnipeg, Man.

TABLE 1.—PAIRING IN INTERSPECIFIC AND INTERGENERIC F_1 HYBRIDS

Species or cross	2n	Pairing in hybrids		Literature reference	Kihara's formula
		Range	Usual		
T. durum Desf.	28				AB
T. dicoccum Schubl.	28				AB
T. vulgare Vill.	42				ABD*
Ae. triuncialis L.	28				C^uC
Ae. variabilis Eig	28				C^uS^v
Ae. ovata L.	28				C^uM^o
Ae. ventricosa Tausch	28				DM^v
Ae. triuncialis × *T. dicoccum*	28	1– 7	4–5	6	
Ae. triuncialis × *T. vulgare*	35	0– 5	0–3	1, 6, 14	
Ae. ovata × *Triticum* species	28, 35	0– 7	0, 1	1, 2, 4, 6, 14	
T. vulgare × *T. durum*	35	10–14	14	1, 11	
T. vulgare × *T. dicoccum*	35	9–14	14	11	
T. dicoccum × *T. durum*	28	12–14	14	1, 20	

* It should be noted that throughout this study the symbol D, first named by Kihara (5), is used to designate the third genome of *T. vulgare.* The symbol C is commonly used synonymously by many workers but in the strict sense can be applied only to certain of the *Aegilops* species.

(= *T. aestivum* L.) (8, 12). No cytological analyses of interspecific F_1 hybrids involving *Ae. juvenalis* have been reported. Neither are there reports of hybrids between this species and *Triticum.*

A great many investigations have been made on the remaining species used in this study. Pertinent data concerning these species and hybrids are presented in Table 1. No mention will be made of the numerous crosses studied by Kihara (9) from which his genome formulae were derived.

Kihara (6) proved quite conclusively that the amount of pairing in a hybrid is not affected by the direction in which the cross is made. However, he found that there were seasonal variations in the number of bivalents formed. This was confirmed by Sears (15) who demonstrated that the maximum pairing of hybrids may not be expressed in certain seasons, thereby leading to variations in pairing from year to year.

MATERIALS AND METHODS

A total of 13 interspecific and intergeneric hybrids were obtained from crosses involving three species of *Tricicum* and five of *Aegilops.* These were as follows:

Ae. juvenalis × *T. vulgare*
Ae. juvenalis × *T. durum*
Ae. juvenalis × *Ae. variabilis*
Ae. juvenalis × *Ae. ovata*
Ae. juvenalis × *Ae. ventricosa*
T. vulgare × *Ae. variabilis*
T. vulgare × *Ae. ovata*
T. vulgare × *Ae. triuncialis*
T. vulgare × *T. durum*
T. vulgare × *T. dicoccum*
T. dicoccum × *Ae. ovata*
Ae. triuncialis × *T. dicoccum*
T. dicoccum × *T. durum.*

Whole spikes of parents and hybrids were collected and fixed in Carnoy's (6:3:1:) solution for cytological examination of pollen mother cells. Fixed material was stored in a refrigerator until examinations could be made. Cytological investigations were conducted on all parent species and F_1 hybrids. Slides were prepared of pollen mother cells at metaphase I of meiosis using the aceto-carmine smear method described by Smith (18). An attempt was made to study at least 200 pollen mother cells of each hybrid. In cases where material was limited, all available cells were counted. A record was kept of the number of univalents, open and closed bivalents and multiple associations for each cell.

RESULTS

All parent species studied showed almost completely normal pairing at metaphase I of meiosis (e.g., Figure 1). In *Ae. ventricosa* a tendency toward asynapsis was observed. A low percentage of cells had two or four univalents present.

Pairing in the three interspecific *Triticum* crosses proved to be as expected. In the cross *T. vulgare* × *T. dicoccum*, there were usually $14^{II}7^{I}$ at metaphase I with occasionally $13^{II}9^{I}$ or $12^{II}11^{I}$. Similar pairing was observed in *T. vulgare* × *T. durum* hybrids. In hybrids of *T. dicoccum* × *T. durum*, most commonly 14^{II} occurred (Figure 2). A low frequency of cells had up to four univalents. These results are in accordance with those of numerous workers (1, 7, 11, 19, 20).

The data concerning the pairing in the remaining 10 interspecific and intergeneric F_1 hybrids involving *Aegilops* and *Triticum* are presented in Tables 2 and 3. In Table 3 the average number of bivalent associations is based on the premise of Kihara (6) that a trivalent is equivalent to one bivalent association and a quadrivalent to two bivalent associations. In Figures 3-8, typical metaphase I plates for certain of these hybrids can be seen.

DISCUSSION

Significance of Pairing

Since there is apparently considerable homoeology between a great number of species within the Triticinae, it is difficult to determine accurately the nature of the pairing observed in interspecific and intergeneric hybrids. Even within a haploid where normally no synaptic mates are present, usually some pairing is observed. This may be accounted for by intergenomic or intragenomic homoeology. O'Mara (13) points out "the possibility also exists that some seemingly homologous association between non-homologous chromosomes may actually be an expression of a tendency to random and irrelevant pairing which manifests itself when a true homology cannot be realized". Thus it seems that pairing can be intergenomic, intragenomic or irrelevant.

There seems to be little doubt that where closed bivalents are regularly observed, the pairing is between chromosomes of a fairly close relationship. Furthermore, where a chiasma can be clearly seen, this should be indicative of at least partial homology. Of lesser significance probably, is the occurrence of stretched open bivalents with no visible evidence of a chiasma. Chromosomes may unite end-to-end merely by strong terminal attractions

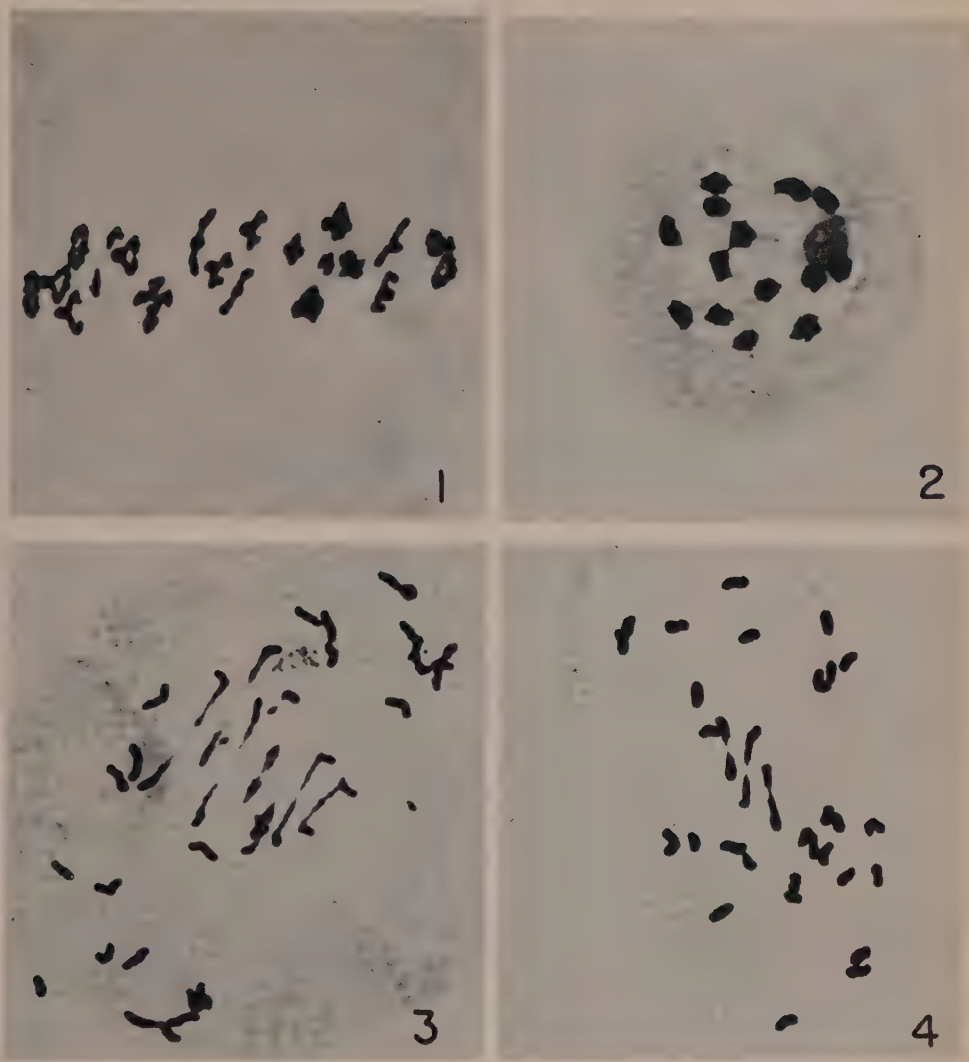


Figure 1. *Ae. juvenalis.* 21^{II}.
Figure 2. *T. dicoccum* × *T. durum.* 14^{II} (Late diakinesis).
Figure 3. *Ae. juvenalis* × *T. vulgare.* 6^{II} (2 closed, 4 open) 1^{III} 27^{I}.
Figure 4. *Ae. juvenalis* × *T. durum.* 3^{II} (open) 29^{I}.
Approximately 600X

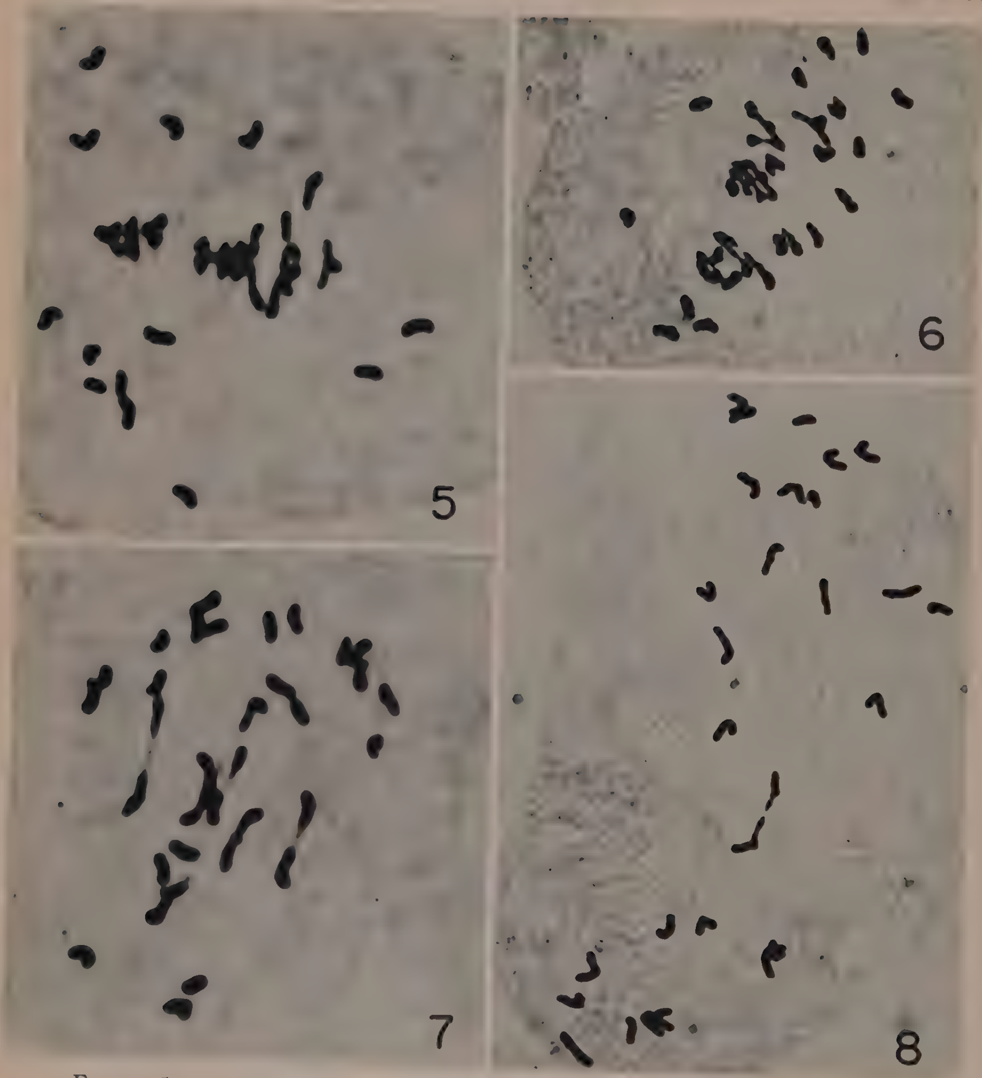


FIGURE 5. *Ae. juvenalis* × *Ae. variabilis.* 10^{II} (7 closed, 3 open) 15^{I}.
FIGURE 6. *Ae. juvenalis* × *Ae. ovata.* 5^{II} (1 closed, 4 open) 3^{III} 16^{I}.
FIGURE 7. *T. vulgare* × *Ae. variabilis.* 6^{II} (open) 23^{I}.
FIGURE 8. *T. dicoccum* × *Ae. ovata.* 1^{II} (open) 26^{I}.

Approximately 600X

TABLE 2.—CHROMOSOME ASSOCIATIONS IN THE INTERSPECIFIC AND INTERGENERIC F_1 HYBRIDS

Hybrid	2n	Number of cells examined	Univalents per cell		Closed bivalents per cell		Open bivalents per cell		Trivalents per cell		Quadrivalents per cell	
			Range	Average	Range	Average	Range	Average	Range	Average	Range	Average
Ae. juvenalis × *T. vulgare*	42	266	16–34	25.88	0–4	1.23	3–11	5.53	0–3	0.78	0–1	0.06
Ae. juvenalis × *T. durum*	35	264	19–35	27.30	0–1	0.11	0–8	3.33	0–2	0.27	0–1	0.004
Ae. juvenalis × *Ae. variabilis*	35	207	3–21	12.48	1–7	4.40	3–13	5.13	0–3	1.03	0–1	0.09
Ae. juvenalis × *Ae. ovata*	35	204	10–23	16.76	0–5	2.48	3–11	5.01	0–4	1.00	0–1	0.07
Ae. juvenalis × *Ae. ventricosa*	35	64	16–31	22.78	0–2	0.63	2–9	4.67	0–1	0.44	0–1	0.08
T. vulgare × *Ae. ovata*	35	79	24–33	29.15	0	0.00	1–5	2.70	0–1	0.15	0	0.00
T. dicoccum × *Ae. ovata*	28	97	18–28	25.16	0	0.00	0–5	1.42	0	0.00	0	0.00
T. vulgare × *Ae. variabilis*	35	380	13–31	21.89	0–1	0.17	1–11	5.82	0–3	0.38	0	0.00
T. vulgare × *Ae. triuncialis*	35	247	11–33	24.09	0–1	0.04	1–12	5.10	0–2	0.15	0–1	0.04
Ae. triuncialis × *T. dicoccum*	28	75	8–22	16.17	0	0.00	3–10	5.57	0–1	0.23	0	0.00

TABLE 3.—PAIRING FREQUENCIES IN THE INTERSPECIFIC AND INTERGENERIC F_1 HYBRIDS

Hybrid	Total Number of Cells	Number of pollen mother cells with bivalent associations*																	Mean bivalent association	Standard error of mean
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
Ae. juvenalis × *T. vulgare* (2n = 42)	266					7	14	37	67	58	55	19	6	3					7.67	± .10
Ae. juvenalis × *T. durum* (2n = 35)	264	9	14	29	56	71	58	23	3	1									3.72	± .09
Ae. juvenalis × *Ae. variabilis* (2n = 35)	207								2	8	34	50	52	34	18	6	2	1	10.76	± .11
Ae. juvenalis × *Ae. ovata* (2n = 35)	204							4	32	54	71	34	8	1					8.62	± .08
Ae. juvenalis × *Ae. ventricosa* (2n = 35)	64			2	8	5	12	11	10	11	5								5.89	± .24
T. vulgars × *Ae. ovata* (2n = 35)	79		6	27	27	11	8												2.85	± .12
T. dicoccum × *Ae. ovata* (2n = 28)	97	8	53	28	4	3	1												1.42	± .09
T. vulgare × *Ae. variabilis* (2n = 35)	380			3	6	35	54	105	96	55	18	7	1						6.37	± .08
T. vulgare × *Ae. triuncialis* (2n = 35)	247		2	1	23	52	56	59	30	18	3	2		1					5.38	± .10
Ae. triuncialis × *T. dicoccum* (2n = 28)	75				2	10	24	19	10	6	3	1							5.80	± .17

* Trivalent = 1 bivalent association.
Quadrivalent = 2 bivalent associations.

(Figure 4). At metaphase the united regions stretch to form a tapered end, typical of an open bivalent, but with no actual chiasma visible. Such cases can hardly be considered as true bivalents but could be a type of secondary pairing which may or may not be an indication of segmental homology.

The following hypotheses have been accepted as a basis for the genome homologies established in this study:

1. Where closed bivalents occur regularly, true homology must exist.
2. Up to three or four bivalents may be accounted for through homoeologous pairing.
3. Where seven or more pairs occur in a high frequency of cells, one genome is likely to be common to both parents.

Pairing in the Hybrids Under Study

In the cross *Ae. juvenalis* × *T. vulgare*, the range in bivalents per cell was from 4 to 12 with an average of 7.67. In these cells there were up to 4 closed bivalents. From these data it is concluded that the above species have one genome in common. Since pairing between 7 chromosomes of the two constituent species was not always complete and regular, it may be assumed that some chromosomal changes have occurred within one or both species during their evolution. Such changes would prevent complete compatibility in pairing at the present time. The fact that an average of only 1.23 closed bivalents occurred per cell also supports this hypothesis.

On the basis of the *juvenalis-vulgare* cross alone, it can be hypothesized that *Ae. juvenalis* has either the A, B or D genome in its chromosome complement, but the actual genome involved cannot be determined. However, when *Ae. juvenalis* was crossed with *T. durum* which has only the A and B genomes, the average bivalent frequency was 3.72 per cell and only rarely was there a closed bivalent. The amount of pairing observed in this hybrid can be accounted for on the basis of homoeologous associations. These two species, therefore, do not have a genome in common. Consequently, the pairing observed in the *Ae. juvenalis* × *T. vulgare* hybrid must have been between chromosomes of the D genome and this genome must be common to both species. This cytological study, therefore, supports the morphological evidence used by previous investigators to place *Ae. juvenalis* in the same taxonomical section as *Ae. squarrosa* which has the D genome.

In the cross *Ae. juvenalis* × *Ae. variabilis* there was an average of 10.76 bivalents per cell. Of these, 4.40 were closed bivalents, signifying considerable allosyndetic pairing. The genomes of *Ae. variabilis* have been previously identified as C^uS^v. From the amount and kind of pairing in the above hybrid, it is concluded that *Ae. juvenalis* also carries at least one of these genomes.

Ae. ovata has the genomes C^uM^o. Studies of *Ae. juvenalis* × *Ae. ovata* showed an average of 8.62 pairs per cell with 2.48 closed bivalents per cell. These data are indicative of a single genome common to the two parent species.

The genome homologies observed in the above two crosses suggest that *Ae. juvenalis* has the C^u genome since both *variabilis* and *ovata* have

this genome. The higher pairing frequency in the cross with *variabilis* may either be indicative of partial homology with the S^v genome or of a tendency for the *variabilis* chromosomes to pair in the absence of true homologues.

Pairing in the *Ae. juvenalis* × *Ae. ventricosa* hybrid proved to be the most erratic and least conclusive in the entire study. An average of 5.89 bivalents per cell was observed with only 0.63 closed ones. Although Kihara (9) has suggested that *Ae. ventricosa* has the D genome, Sears (16) earlier demonstrated that it is not homologous to the D of *T. vulgare* but that it has considerable homoeology with it. The present investigation indicates that the D of *Ae. ventricosa* is not completely homologous with that of *Ae. juvenalis* since a relatively low number of bivalents was observed.

In the hybrids *T. vulgare* × *Ae. ovata* and *T. dicoccum* × *Ae. ovata*, only 2.85 and 1.42 pairs per cell, respectively, were observed. All were open bivalents indicating no real homology between species.

Somewhat less easily explained is the high average frequency of bivalents (6.37) in the *T. vulgare* × *Ae. variabilis* hybrid. Since these parents reportedly do not have any genomes in common the pairing must be attributed to autosyndesis particularly since a closed bivalent was rarely observed. Furthermore, since the *vulgare-ovata* hybrid gave such a low number of bivalents, the pairing in the *vulgare-variabilis* cross most likely resulted mainly from autosyndesis within or between the *variabilis* genomes. Supporting evidence for such a hypothesis is the high number of bivalents observed in *Ae. juvenalis* × *Ae. variabilis* (10.76) as compared with the number found in *Ae. juvenalis* × *Ae. ovata* (8.62). These data would suggest that at least four *variabilis* chromosomes have an affinity for forming two pairs when their normal homologues are absent. Possibly the C^u and S^v genomes are more closely related than their symbols would indicate.

The intergeneric crosses *T. vulgare* × *Ae. triuncialis* and *Ae. triuncialis* × *T. dicoccum* proved to be in somewhat the same category as the *vulgare-variabilis* cross in regard to pairing behaviour. These hybrids had 5.37 and 5.80 bivalents per cell, respectively. Only rarely in the *vulgare-triuncialis* hybrid and never in the *trincialis-dicoccum* cross was a closed bivalent observed. Since *Ae. triuncialis* has the genomes C^uC, the C^u supposedly having been modified from the C, the pairing in these hybrids may be largely attributed to homoeology between the *triuncialis* chromosomes. Sears (17) however, points out that the cytological and morphological data show no closer relationship of C to C^u than C to the M of *Ae. comosa* and concluded that Kihara's formulae tend to overemphasize the closeness of this relationship. The present study indicates that some homoeology exists between certain chromosomes of *Ae. triuncialis*. In both crosses studied the average pairing was almost identical. If the number of *Triticum* chromosomes present had a major influence on the number of bivalents formed, a marked reduction would have been noted in the *triuncialis-dicoccum* hybrid.

CONCLUSIONS

Although the pairing frequency in the hybrid under study proved to differ considerably from cell to cell in the same cross, such variability

could be expected on the basis of incomplete chromosome homology, environmental effects and the natural variability which is found in all hybrid material. Most previous investigators have demonstrated that instability in pairing usually accompanies wide crosses. The use of polyploid species as parents in this study probably increased the difficulty of establishing clear-cut relationships. Nevertheless, the data presented herein strongly suggest that the C^u and D genomes are present in *Ae. juvenalis*. There was no definite evidence as to the identity of the third genome in *Ae. juvenalis* from the crosses studied.

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to R. F. Peterson, Cereal Breeding Laboratory, Winnipeg, for his many helpful suggestions throughout this study; and to W. E. Clark, Plant Pathology Laboratory, Winnipeg, for the photographic plates.

REFERENCES

1. Aase, H. C. Cytology of *Triticum*, *Secale*, and *Aegilops* hybrids with reference to phylogeny. Research Studies, State College of Washington 2 : 1-60. 1930.
2. Bleier, H. Zytologische Untersuchungen an seltenen Getreideund Rübenbastarden. Zeit. ind. Abst. u. Vererbgsl., Suppl. 1 : 447-452. 1928.
3. Eig, A. Monographische—kritische Übersicht der Gattung *Aegilops*. Repert. Spec. nov. Regn. veg. Beith. 55 : 1-228. 1929.
4. Kagawa, F. Cytological studies on the pollen formation of the hybrids between *Triticum* and *Aegilops*. Jap. J. Bot. 4 : 345-360. 1929.
5. Kihara, H. Zytologische und genetische Studien bei wichtigen Getreide-Arten mit besonderer Rücksicht auf das Verhalten der Chromosomen and die Sterilität in den Bastarden. Mem. Coll. Sci. Kyoto Imp. Univ. 1 : 1-200. 1924.
6. Kihara, H. Conjugation of homologous chromosomes in the genus hybrids *Triticum* × *Aegilops* and species hybrids of *Aegilops*. Cytologia 1 : 1-15. 1929.
7. Kihara, H. Cytogenetics of species hybrids. Curr. Sci. Suppl. Spec. No. on Genetics : 20-23. 1938.
8. Kihara, H. Die Entdeckung des DD-Analysators beim Weizen. Vorläufige Mitteilung. Agr. and Hor. 19 : 889-890. 1944.
9. Kihara, H. Genomanalyse bei *Triticum* und *Aegilops*. IX. Systematischer Aufbau der Gattung Aegilops auf genomanalytischer Grundlage. Cytologia 14 : 135-144. 1949.
10. Kihara, H. Considerations on the evolution and distribution of *Aegilops* species based on the analyser-method. Cytologia 19 : 336-357. 1954.
11. Love, R. M. Chromosome behaviour in F_1 wheat hybrids. I. Pentaploids. Can. J. Res., *C*, 19 : 351-369. 1941.
12. McFadden, E. S., and E. R. Sears. The artificial synthesis of *Triticum spelta*. Records Gen. Soc. Am. 13 : 26-27. 1944.
13. O'Mara, J. G. The cytogenetics of Triticale. Bot. Rev. 19 : 587-605. 1953.
14. Percival, J. Cytological studies of some hybrids of *Aegilops* sp. × wheats, and of some hybrids between different species of *Aegilops*. J. Genet. 22 : 201-278. 1930.
15. Sears, E. R. Chromosome pairing and fertility in hybrids and amphidiploids in the *Triticinae*. Mo. Agr. Expt. Sta. Res. Bull. 337. 1941.
16. Sears, E. R. The amphiploids *Aegilops cylindrica* × *Triticum durum* and *Ae. ventricosa* × *T. durum* and their hybrids with *T. aestivum*. J. Agr. Res. 68 : 135-144. 1944.
17. Sears, E. R. The cytology and genetics of the wheats and their relatives. Adv. Genet. 2 : 239-270. 1948.
18. Smith, Luther. The acetocarmine smear technic. Stain Tech. 22 : 17-31. 1947.
19. Thompson, W. P. The causes of the cytological results obtained in species crosses in wheat. Can. J. Res., *C*. 10 : 190-198. 1934.
20. Thompson, W. P., and H. T. Robertson. Cytological irregularities in hybrids between species of wheat with the same chromosome number. Cytologia 1 : 252-262. 1930.

TRENDS OF COMMON ROOT ROT OF WHEAT IN SASKATCHEWAN[1]

B. J. Sallans[2]

Plant Pathology Laboratory, Saskatoon, Saskatchewan

[Received for publication July 7, 1955]

ABSTRACT

Surveys indicate that common root rot (*Helminthosporium sativum* and *Fusarium* spp.) of wheat has increased over a 20-year period in Saskatchewan. A study of the average yields of wheat over the period revealed that there is a relation between incidence of disease and the factors that influence growth. Conditions which resulted in average yields of 16 to 20 bushels per acre were least favourable for development of root rot. Yields below 16 bushels per acre were accompanied by marked increases in the disease. A less marked though similar increase in root rot occurred when yields were over 20 bushels per acre. Areas with high percentages of crop land in wheat, barley or rye had high degrees of root rot infection in wheat.

INTRODUCTION

Data have been recorded in annual plant disease surveys over a number of years on the incidence of common root rot of wheat caused by *Helminthosporium sativum* P.K. & B. and *Fusarium* spp. The records have been examined for the 20-year period from 1934 to 1953 and trends have been noted. These trends with a discussion of various factors influencing them form the subject of this paper.

ORIGIN AND NATURE OF DATA

In a paper which appeared in 1948 the author presented a statistical study (8) of the relation of common root rot and other factors to the yields of wheat during the first 10-year period of the 20 years covered in this study. Figures were given for the nine crop-reporting districts as used by the Secretary of Statistics of the Saskatchewan Government Department of Agriculture (1). These districts served as units in the present work because they represent widely diverse climatic areas with differing cropping practices, and they are convenient also in that acreage and production data are available.

Common root rot data have been gathered in such a way that twenty or more fields were sampled for each crop district. Some exceptions to this rule occurred when fewer than twenty, rarely as low as ten, fields were examined in a crop district. An adequate random sampling of fields over an area the size of Saskatchewan is not possible in a limited plant disease survey. However, since the districts were usually traversed by the same roads year by year, it is believed that the data will bear comparison within crop districts.

Root rot was assessed on the basis of incidence and severity of lesions of the subcrown internodes, and disease ratings were calculated by the method given by Russell and Sallans (7).

[1] Contribution No. 1449 from Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.
[2] Senior Plant Pathologist.

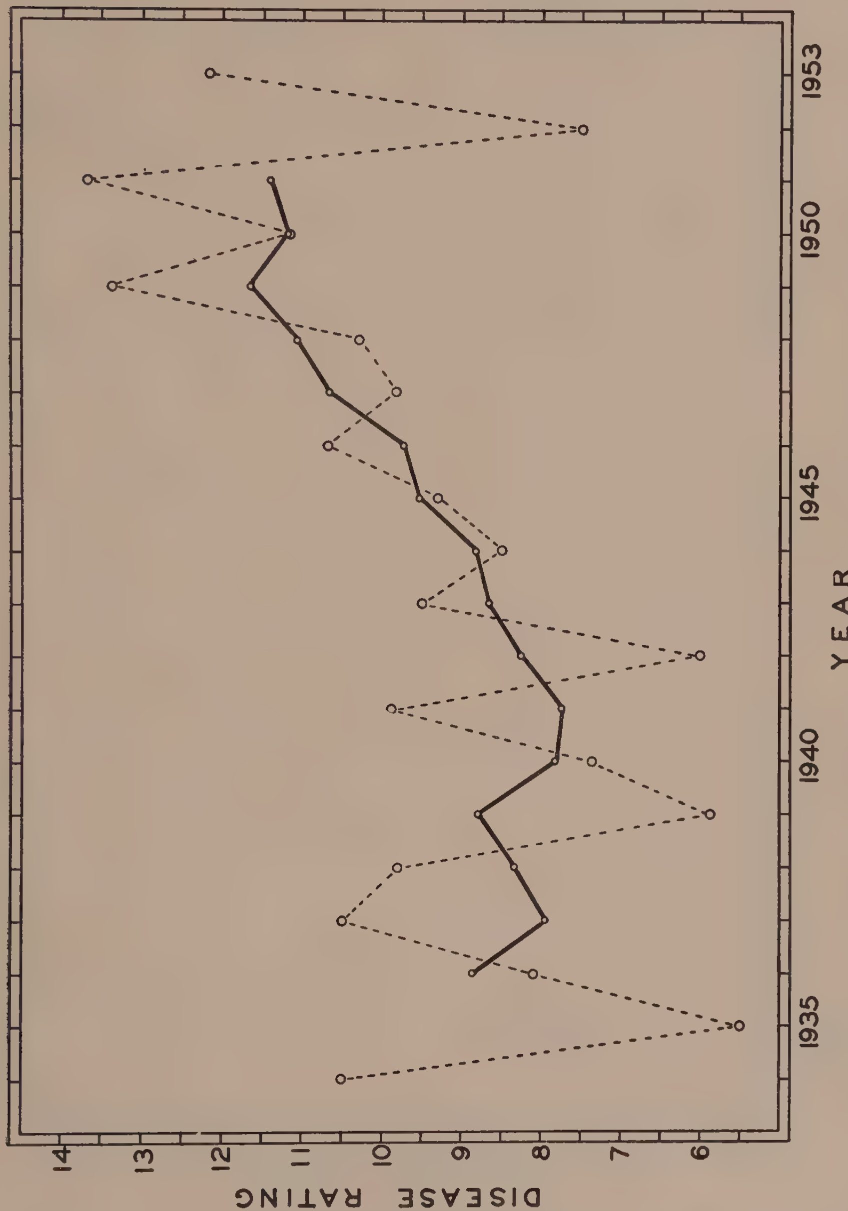


FIGURE 1. Trend in disease ratings for common root rot of wheat in Saskatchewan for the 20-year period 1934 to 1953. Annual fluctuations are indicated by the broken line. The solid line shows the 5-year moving average, the points being plotted for the middle of each 5-year period.

TRENDS IN COMMON ROOT ROT

The average disease ratings for the province as a whole are assembled into a graph for the 20-year period 1934 to 1953 (Figure 1). The annual variations (*broken line*) are quite large and tend to obscure the main trend of the data. To indicate the trend more clearly a second line (*solid*) depicting a 5-year moving average has been superimposed on the graph of Figure 1. This line indicates little change in the disease during the period 1936-40. From 1941 to 1949 the line is almost straight and shows a marked increase in the disease. However, it levels off for the period 1950-51, during which no increase in the disease occurred. The very low incidence of root rot in 1952 may be largely responsible for this second horizontal section of the trend line.

The significance of the increase in common root rot indicated by the trend lines of Figure 1 requires some consideration. This is especially desirable since data were accumulated over a period of years, and may best be done by examination of the trends within the nine crop districts individually.

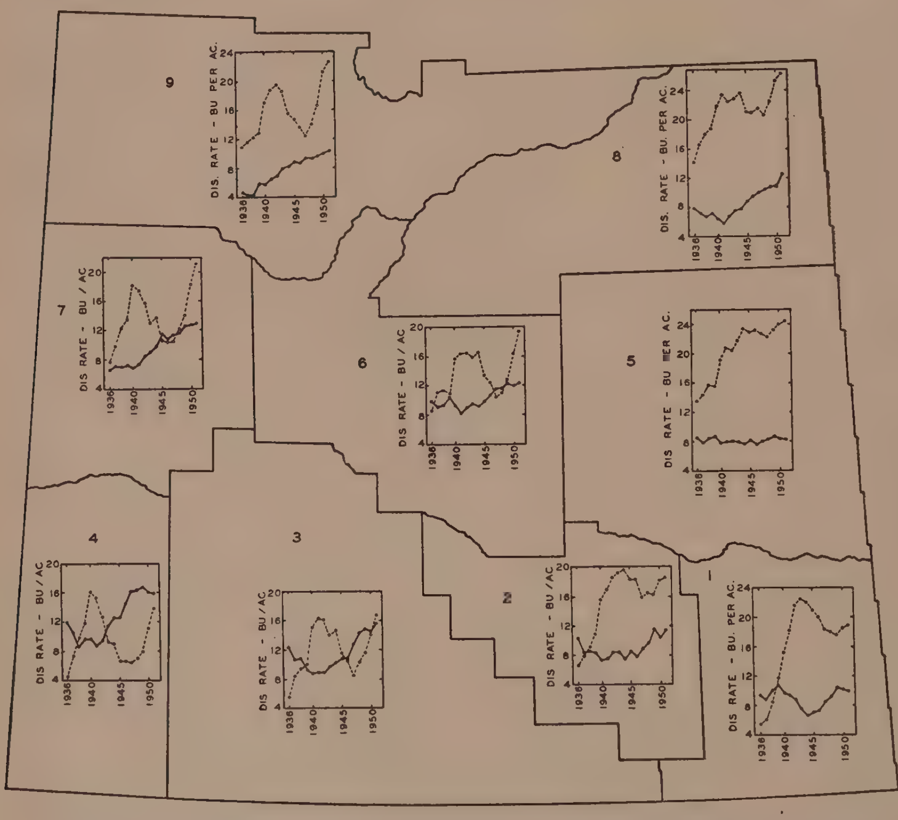


FIGURE 2. Outline of the agricultural portion of Saskatchewan showing boundaries of the crop-reporting districts which are numbered 1 to 9. The chart for each crop district shows 5-year moving average trend lines for yields of wheat (broken lines) and for common root rot (solid lines). The ordinate scales apply to both yields of wheat in bushels per acre and disease ratings for common root rot. The abscissa scale shows the mid-points of the 5-year periods which were averaged.

The trend lines for the individual crop districts are shown in Figure 2 in relation to an outline map of the southern, agricultural portion of the province. The trend lines show disease ratings based on 5-year moving averages. Included in the same charts are trend lines for yields of wheat based on 5-year moving averages for the same period of 20 years. An examination of the trend lines shows that their slopes are related to the geographic location of the crop districts. Apparently root rot has not increased in crop districts 1 and 5 in the southeastern and eastern sections of the province. On the other hand, in the western crop districts, 4, 7, and 9, the slopes of the trend lines indicate considerable increases in root rot. In the intervening crop districts of central and southern Saskatchewan the slopes of the lines indicate that disease increases are intermediate between the eastern and western areas. Thus the non-uniform slopes of the trend lines may be taken as evidence that the disease has been increasing in some areas and the probability is that there has been an actual increase in the western crop districts.

There is a strong suggestion in Figure 2 of cyclical trends in yields of wheat, which are particularly marked in western and central crop districts but evident in all districts. Of course the period of 20 years covered in this study is much too short to form the basis for predictions of yields. Using data from Winnipeg, Regina, and Edmonton in Western Canada, Currie (4) has shown the close relation of high and low periods of rainfall and minimum and maximum periods of sun spot activity over a period of seven solar cycles. It is interesting to note, then, that the periods of maximum yields centring on the years 1941-42 and 1952-53 correspond with periods of minimum sun spot activity; and periods of minimum yields, 1936-37 and 1947-48 with periods of maximum sun spot activity.

The data charted in Figure 2 show a fairly regular increase in root rot ratings for the period 1936 or 1938 to 1951 in the western crop districts, namely districts 4, 7, and 9. On the eastern side of Saskatchewan, in crop district 5, the disease ratings remained at a very uniform level. The disease ratings for crop districts 1, 2, 3, 6, and 8 show a net downward trend initially which is followed by a reversal of the trend upward. In crop districts 1, 2, 3, and 6 and to a lesser extent in districts 4, 7 and 8 there appears to be a negative relation between yields of wheat and disease rating up to about the year 1946. In crop district 3, for example, this relationship is fairly close. From about 1946 to 1951, however, the upward trend for disease in most districts coincides with an upward trend in yields of wheat. Thus though the data for the first parts of the curves in most of the districts suggest that common root rot varies inversely as the yield of wheat, this suggestion is not borne out in the period 1946 to 1951 especially in the western and central districts. The practice of maintaining straw and stubble on the surface of the soil which became established in Saskatchewan during the period under study may have an important bearing on the increase of root rot during the years 1946 to 1951. This possibility is discussed later in this paper.

REGIONAL VARIATION IN ROOT ROT

The curves in Figure 2 indicate that there were regional differences in the incidence and increase of common root rot. In crop district 5,

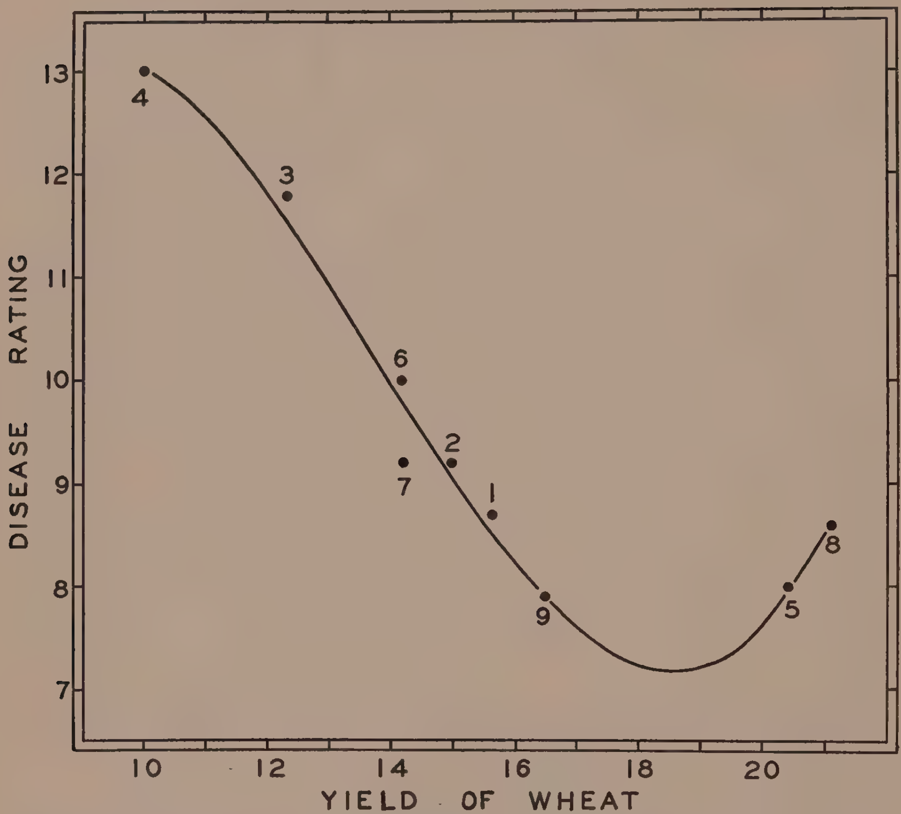


FIGURE 3. Curvilinear relation between disease rating and yield of wheat in bushels per acre. The annual data for the 20-year period 1934 to 1953 are averaged for each crop district, numbered 1 to 9 as in Figure 2.

particularly, variations in the disease were very minor, though during the years from 1940 to 1947 yields have greatly improved over the early years of the period under study.

In Figure 3 the 20-year average yield of wheat in bushels per acre for each crop district and the average root rot rating of wheat over the same period, 1934 to 1953, are graphically represented. The relationship here is not necessarily one of cause-and-effect since both variables are related to the group of many factors which influence the growth of wheat plants. Yield has been taken here as the end result of these factors which are too numerous and insufficiently measured to be graphically related to root rot incidence. Consequently, for the purpose of Figure 3, yield has been taken as the independent variable in as much as it is a measure of all growing conditions. Though root rot is one of the factors influencing yield, it has been taken in this figure as the dependent variable, in order to bring out its dependence upon other factors in plant growth. That common root rot is related to some of these factors, such as rainfall, has been shown in previous studies (8). The graph of Figure 3 indicated that in crop districts where yields are generally low root rot ratings are generally

high, and vice versa. With the exception of crop districts 5 and 8 there is a straight-line relationship. Obviously, however, the data for crop districts 5 and 8 cannot be reconciled with those of the other districts on the basis of a straight-line relationship. A somewhat better approximation of the data appears in the second degree polynomial

$$Y = 31.93 - 2.51X + 0.066X^2$$

where Y is disease rating and X is yield. The third degree polynomial

$$Y = -12.80 + 6.897X - 0.5688X^2 + 0.01375X^3$$

very closely approximates the actual disease ratings.

Over the period under study *Helminthosporium sativum* was found to be the most important cause of root rot in the relatively dry crop districts of central and western Saskatchewan. In the same areas *Fusarium* spp. are relatively unimportant. An interesting possibility, which may account for the importance of common root rot in crop districts 8 and 5, is that conditions which favour the good crops of these districts also favour infections by *F. culmorum* and other *Fusarium* spp. In 1949 widespread infections of wheat by *Fusarium* spp. occurred in crop district 8. Evidence is not available, however, to show the relative importance of *Fusarium* spp. and *H. sativum* in crop districts 5 and 8 over the whole period of 20 years.

Two workers have shown regional variation in the incidence of *H. sativum* and *Fusarium* spp. in the Prairie Provinces. In reporting isolations from crowns of wheat plants Broadfoot (3) found that *F. culmorum* isolates outnumbered those of *H. sativum* at Olds, Alberta, but that *H. sativum* was predominant at Morden in Manitoba, Indian Head, Swift Current and Scott in Saskatchewan, and Lethbridge and Vermilion in Alberta. Tyner* reported that more colonies of *H. sativum* than of *F. culmorum* were obtained from wheat stubble from central Alberta. On the other hand, *F. culmorum* was the predominant fungus from stubble from the Peace River area. He found, moreover, that most of the cultures of *F. culmorum* from the latter area were very pathogenic to wheat seedlings in the greenhouse. Neither Broadfoot nor Tyner suggests that growing conditions, such as moisture supply, influence the relative importance of *H. sativum* and *F. culmorum* as causes of common root rot. Though it is reasonably clear that *H. sativum* is important under poor crop growing conditions, it remains to be shown whether *F. culmorum* is favoured by factors necessary for good crops.

CROPPING PRACTICE AND ROOT ROT

Although wheat, barley and rye are the predominant crops in some districts, they comprise a smaller proportion of the crops in districts 1, 5 and 8. Data were assembled from the reports of the Secretary of Statistics** (1) on acreages of various crops. From these data were calculated the percentages of crops susceptible to common root rot, namely wheat, barley, and rye. In a comparison of crop districts, Figure 4 shows a straight-line relationship between the percentage of crop susceptible to

* Tyner, L. E. *Private communication.*
** Evans, Edward H. Secretary of Statistics, Dept. of Agriculture, Regina, Sask. *Private communication.*

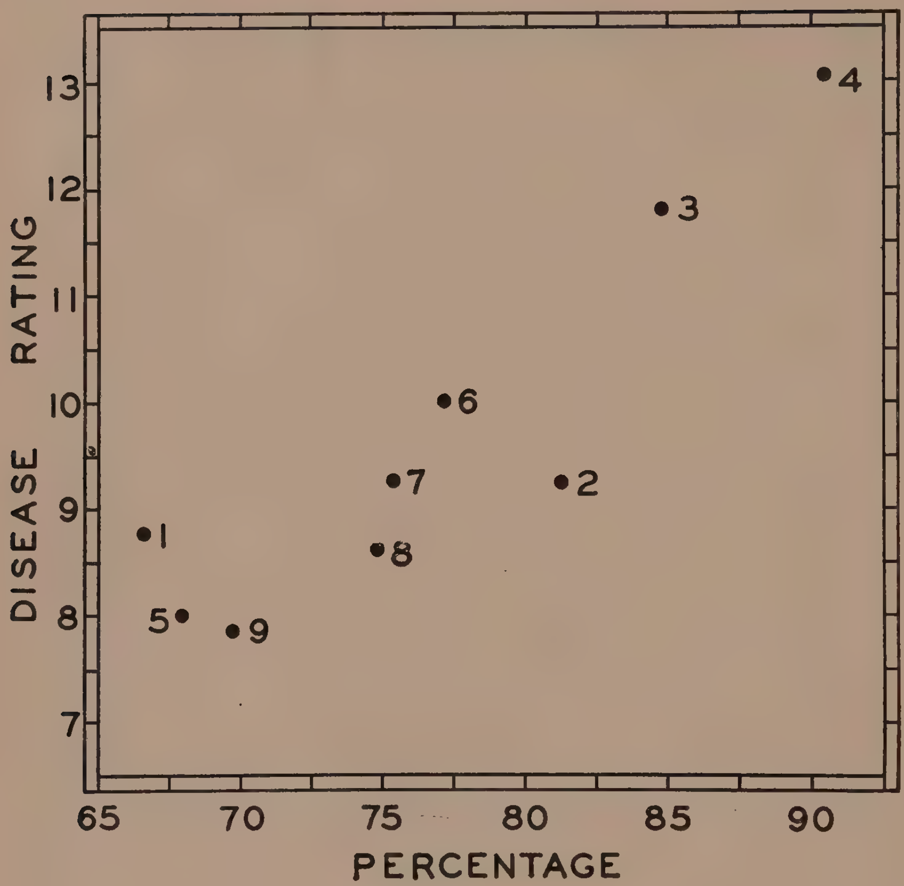


FIGURE 4. Correlation between disease ratings and percentages of the crop that are susceptible to common root rot fungi (those attacking wheat, barley and rye). The data for the 19-year period 1934-1952 for crop districts 1 to 9 are averaged. The correlation coefficient 0.904 is significant at the 1 per cent level.

the root disease fungi and the disease ratings. Variation from the straight line for crop district 1 may be partly due to reduction in wheat acreage as a result of several stem rust epidemics. Whether the relatively high disease ratings of crop districts 4 and 3 are the result of seeding 91 and 85 per cent respectively of the crop land to susceptible wheat, barley or rye, is not clear. That such may be the case is indicated by the results obtained by Broadfoot (2) in rotation experiments. In his tests root rot was much less severe in wheat after oats or summer fallow than after wheat or barley. In the present studies fallow probably is not the cause of the variation in root rot infection in the crop districts of Saskatchewan, because approximately one-third of the cultivated land in all areas is fallowed each year (5). It may be that a factor in the increase of common root rot over the past several years in crop districts 3 and 4 particularly is the relatively high proportion of susceptible crops grown in those areas as shown in Figure 5.

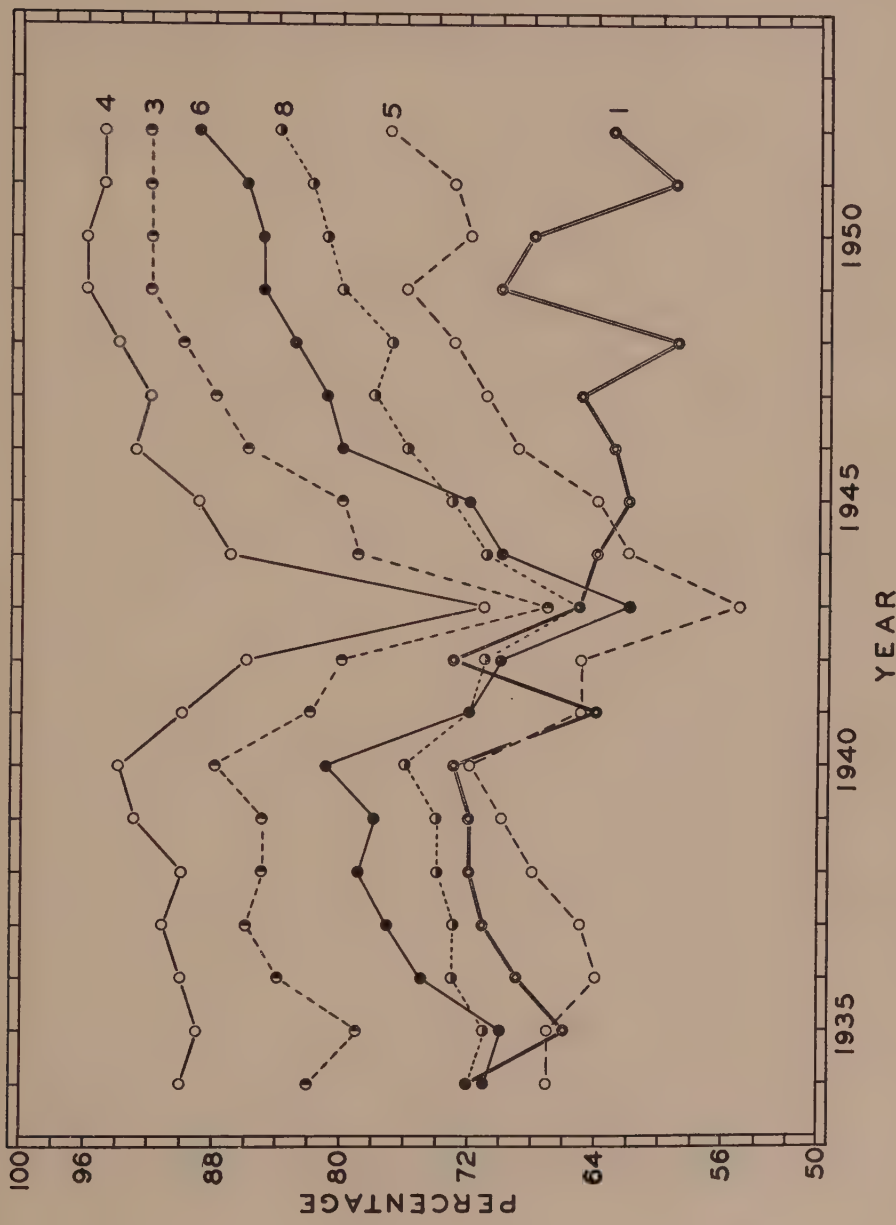


FIGURE 5. Percentage of the total crop in each crop district which is susceptible to *Helminthosporium sativum*. The susceptible crops are wheat, barley and rye. The numbers 1, 3, 4, 5, 6 and 8 refer to crop districts as shown in Figure 2.

There has been considerable variation in cropping practices over the 20-year period covered by this study. This variation is due to acreage limitations that followed the huge surpluses of wheat during the period 1941-43 and to the emphasis on livestock production of the war years. A return to high acreages of wheat and barley occurred during the period 1944-46 in all crop districts (see Figure 5). In each crop district except number 1 the period 1946-52 shows a rather consistent trend towards increased percentages of land sown to wheat and other crops susceptible to root rot when compared with the period 1934-40.

DISCUSSION

Several factors may have influenced the trends observed over the years 1934 to 1953 in common root rot infections of wheat in Saskatchewan. Weather factors, particularly rainfall, appear to have been most important. During good crop periods root rot has either declined or remained relatively static, but during poor crop periods it has tended to increase rather markedly. The dependence of root rot on weather, however, does not appear to account entirely for the apparent upward trends, especially in the period 1946 to 1951.

A second factor influencing root rot is probably the cropping practice which during the period has been modified first to reduce and then to increase the percentage of cropped land in root rot susceptible crops. Likewise the severe rust epidemics of 1935 and 1938 probably tended to discourage wheat growing in crop district 1 and to some extent in districts 5 and 2, and to encourage livestock raising.

A third factor which may be responsible in part for the increase in root rot is the use of the straw and stubble mulch since the dust-bowl conditions of the period 1931-37. Ledingham (6) reports evidence to show that ploughing with the mouldboard plough to turn down crop debris results in lower disease ratings than when the debris is left on the surface as a trash cover. The practice of maintaining a cover of stubble and straw on the soil violates an important principle of disease control, namely, that debris from a diseased crop should be completely ploughed under, burned or removed to prevent it from being the source of inoculum for succeeding crops.

Though the maintenance of a straw mulch on the land was recommended in Saskatchewan as early as 1932 the practice has been followed increasingly only as suitable machinery for this purpose has been evolved to displace the plough. It is perhaps significant that the root rot trend lines of Figure 2 move upward, especially in the western crop districts throughout the later part of the period when the use of trash cover was firmly established as a cultural practice to prevent soil drifting and to return fibre to the soil.

However, it is obvious that conservation of the soil by maintaining a trash cover throughout most of the year, especially in the summer fallow year, must be practised as long as cereals remain the principal crops. It is a common practice in some areas of lighter soils to plough and seed

second crops of wheat or barley after wheat in one operation, so that a stubble cover is maintained during all but a few days before the land is occupied by the next crop of plants.

The application of this practice to first crops of wheat after summer fallow does not appear to be practicable. The mulch cover must be maintained as long as possible. This involves surface tillage, which keeps the crop debris partially above the soil level where inoculum of *H. sativum* increases during successive periods of sporulation. This inoculum is never completely turned under by the cultivator or seeding equipment. Consequently, as shown by survey data over the years, first crops of wheat on summer fallow are infected by root rots at least to the same degree as, and often to a higher degree than, second crops. So far other means of destroying inoculum of *H. sativum*, such as by the use of ground sprays, have not been investigated.

The relationship between common root rot ratings and yields of wheat per acre on the basis of crop districts is undoubtedly related to the differing ecological conditions existing within the various argicultural areas of Saskatchewan. Whether or not species of *Fusarium* are largely responsible for root rot in the more productive crop districts 8 and 5, as suggested above, there appears to be an optimum set of conditions when root rot infections are relatively low. These are conditions which favour an average yield for a crop district over a period of years of 16 to 20 bushels per acre. While conditions resulting in lower yields obviously favour root rot development it is not very clear that circumstances favouring crops of more than 20 bushels per acre also favour root rot development.

REFERENCES

1. Annual reports of the Secretary of Statistics, *in* Annual reports of the Saskatchewan Department of Agriculture for years ending April 30, 1935 to 1953.
2. Broadfoot, W. C. Studies on foot and root rot of wheat. III. Effect of crop rotation and cultural practice on the development of foot rot of wheat. Can. J. Research 10 : 95-114. 1934.
3. Broadfoot, W. C. Studies on foot and root rot of wheat. IV. Effect of crop rotation and cultural practice on the relative prevalence of *Helminthosporium sativum* and *Fusarium* spp. as indicated by isolations from wheat plants. Can. J. Research 10 : 115-124. 1934.
4. Currie, B.W. Climatic trends on the Canadian Prairies. Agr. Inst. Rev. 9 (1) January-February. 1954.
5. Guide to farm practice in Saskatchewan. Dept. of Extension, University of Saskatchewan, Saskatoon, Sask. 1951.
6. Ledingham, R. J. A cultural method for reducing the incidence of common root rot in wheat. Proc. Can. Phytopath. Soc. 21 : 13. 1953.
7. Russell, R. C., and B. J. Sallans. The effect of phosphatic fertilizers on common root rot. Sci. Agr. 21 : 44-51. 1940.
8. Sallans, B. J. Interrelations of common root rot and other factors with wheat yields in Saskatchewan. Sci. Agr. 28 : 6-20. 1948.

INFLUENCE OF AN AUREOMYCIN FEED SUPPLEMENT ON GROWTH AND THRIFT OF DAIRY CALVES AND ON RATION DIGESTIBILITY[1]

V. S. LOGAN[2], V. J. MILES[2], AND G. J. BRISSON[3]

Canada Department of Agriculture

[Received for publication February 22, 1956]

ABSTRACT

Forty Holstein and Ayrshire calves of both sexes were used in this study: two replicates of 16 calves in a feeding trial and two replicates of four calves in a digestibility trial. Management practices were followed to ensure a high standard of sanitation. The control ration (Treatment I) consisted of whole milk, calf starter, and hay. In Treatment II an antibiotic feed supplement (Aurofac A) was added to the milk to provide approximately 12 mg. Aureomycin per 100 lb. of body weight daily during the 12-week experimental period and in Treatment III the supplement was added for the first six weeks only. In Treatment IV the antibiotic supplement was added to the calf starter to provide a daily intake of 12 mg. Aureomycin per 100 lb. of body weight daily. It was found that the antibiotic supplement had no influence on the growth, thrift, and feed consumption of the calves. Also antibiotic supplement did not affect digestibility of the total ration. These results support the belief that under certain good management and feeding practices Aureomycin may not influence growth of dairy calves.

INTRODUCTION

The growth-stimulating effect of Aureomycin administered to dairy calves has been discussed in several reviews (5, 12, 13, 18). Growth response has not been consistent under the different conditions of treatment. Although a number of theories have been advanced, the growth-stimulating effect of antibiotics is not well understood. Reported results indicate that the growth stimulus is associated with age, reduction of scours, and increased feed intake. However, growth responses have been observed when incidence of scours was of minor consequence and when feed consumption was not affected.

The indication that Aureomycin will enhance gains in the early weeks of the calf's life (14, 19) suggests that adding the supplement to the milk might have advantages over mixing it in the starter which is not consumed in appreciable quantity before the fifth or sixth week.

Results with older animals (3) showed that feeding Aureomycin depressed the digestibility of crude fibre and the retention of nitrogen. Although it has been shown that Aureomycin may alter rumen or intestinal microflora (7), responses in weight gains have been observed where there was no apparent effect on total number or types of bacteria in the rumen ingesta (14, 19) or in the feces (20).

The experiment reported here was undertaken to determine the practical advantages, if any, of adding an antibiotic supplement to the milk or to the calf starter fed to young dairy calves and to what extent the age of the calf influences the responses. Digestibility of the diet was also determined.

---

[1]Joint contribution Animal Husbandry Division, Experimental Farms Service and Chemistry Division (No. 307), Science Service, Ottawa.

[2]Animal Husbandman and Assistant Animal Husbandman, respectively, Animal Husbandry Division.

[3]Chemist, Chemistry Division.

EXPERIMENTAL

Feeding Trial

Two replicates of 16 calves each were assigned to the feeding experiment. Male and female calves of the Holstein and Ayrshire breeds were used in the first replicate but only Holstein calves were used in the second replicate. Within 24 hours after birth these calves were randomly allotted to the treatments according to breed and sex. The calves were individually fed in pens previously disinfected, then bedded with wood shavings. The wood shavings were replaced every second day. An effort was made to maintain the stable temperature within the range of 50° to 60° F. The treatments were as follows:—

I. Control.

II. Antibiotic Feed Supplement* (Aureomycin) added to the milk for 12 weeks.

III. Antibiotic Feed Supplement added to the milk for the first 6 weeks only.

IV. Antibiotic Feed Supplement added to the calf starter.

In Treatments II and III, the antibiotic feed supplement was fed in the milk at the rate of 3 gm. per 100 lb. of body weight daily, and in Treatment IV was added to the starter to provide an equivalent daily intake.

Colostrum and whole milk were limited to a daily allowance of 6 lb. during the first week, and thereafter milk was fed at the rate of 10 per cent of body weight. The calves had access to starter (maximum of 4 lb. daily) and to hay ad libitum from the eighth day. The starter contained approximately 18.5 per cent protein. The ingredients, by weight, were as follows:—*ground oats* 50; *bran* 20; *linseed oil meal* 20; *ground soy beans* 5; *ground peas* 5; *bone meal* 1, and *salt* 1. The mixed hay, containing approximately 60 per cent legume and 40 per cent grass, was of mediocre quality. In an attempt to encourage hay and starter consumption, all calves were inoculated at 8 days of age with boluses from adult cows. A vitamin A concentrate was fed at the rate of 15,000 i.u. per calf daily. The calves were weighed at weekly intervals. Records were kept of all feed consumed, general health, and incidence of scours.

Digestibility Trial

Four Holstein and four Ayrshire bull calves were used in the digestibility studies. They were fed on a schedule comparable to that of the calves on the feeding trial to 6 weeks of age. Four of these (two Holsteins and two Ayrshires) received the control diet only and the other four (two of each breed) received an equivalent to 12 mg. of Aureomycin (Aurofac A) per 100 lb. of body weight daily added to the diet. From the beginning of the 7th through the 10th week these calves were restricted to a constant feed intake. The digestibility of the whole ration was determined in a trial consisting of an 8-day sub-period when the feed consumption was recorded to establish a constant feed intake, an 8-day preliminary sub-period, and a 12-day collection period.

*Aurofac A containing at least 1.8 gm. of Aureomycin (chlortetracycline) and 1.8 mg. of vitamin B_{12} per pound.

RESULTS AND DISCUSSION

Feeding Trial

The growth curves for the groups of calves in each replicate are shown in Figure 1, together with the Ragsdale standard (17). Two of the four calves on Treatment II, replicate 2, gained less rapidly than the others. One of these two calves had a mild case of scours during the second week, followed by a slight attack of pneumonia. The other calf appeared healthy in every respect.

The data in Table 1 show that the antibiotic supplementation had no significant effect on the growth of the calves whether the comparisons were made at 6 or 12 weeks of age. Discontinuance of antibiotic feeding at 6 weeks in Treatment III was also without influence on the growth rate of the calves. These observations were supported by a covariance analysis where the results of both replicates were combined.

There was no indication (Table 2) that consumption of hay or starter was affected by the antibiotic feed supplement.

The lack of response observed in this experiment is not in agreement with most published results. The daily intake of Aureomycin, approximately 18 mg., was somewhat low as compared to levels of 45 to 100 mg. used by some workers (10, 11, 16, 19). However, daily intakes of 10 to 15 mg. were found sufficient to produce a growth response in several experiments (1, 2, 4). It appears, therefore, that the level of antibiotic feeding cannot account for the lack of growth response observed in this experiment.

It will be seen in Figure 1 that in all groups except one the growth rates were generally greater than the Ragsdale (17) standard. Furthermore, the incidence of scours was only slight in the control and treated groups. This may have a bearing on the antibiotic feed supplement showing no significant effect on the growth rates. Low grade, non-specific infections have been suggested as an important factor influencing growth

Table 1.—Gains of calves fed an antibiotic (aureomycin) supplement (combined results of replicates 1 and 2)

		I Control	II Supplement in milk for 12 wk.	III Supplement in milk first 6 wk.	IV Supplement in meal to 12 wk.
No. of calves		8	8	8	8
Birth wt.	lb.	89.4	87.5	90.4	89.0
Wt. at 6 wk.	lb.	137.6	133.8	141.9	138.7
6 wk. gain as per cent of birth wt.	%	53.9	52.9	57.0	55.8
Wt. at 12 wk.	lb.	212.8	194.0	211.0	220.6
Gain at 12 wk. as per cent of birth wt.	%	69.0	60.9	66.7	73.9
Gain at 12 wk. as per cent of wk. at 6 wk.	%	54.7	45.0	48.6	59.0

FIGURE 1. GROWTH CURVES OF DAIRY CALVES FED ANTIBIOTIC FEED SUPPLEMENT (AUREOMYCIN)

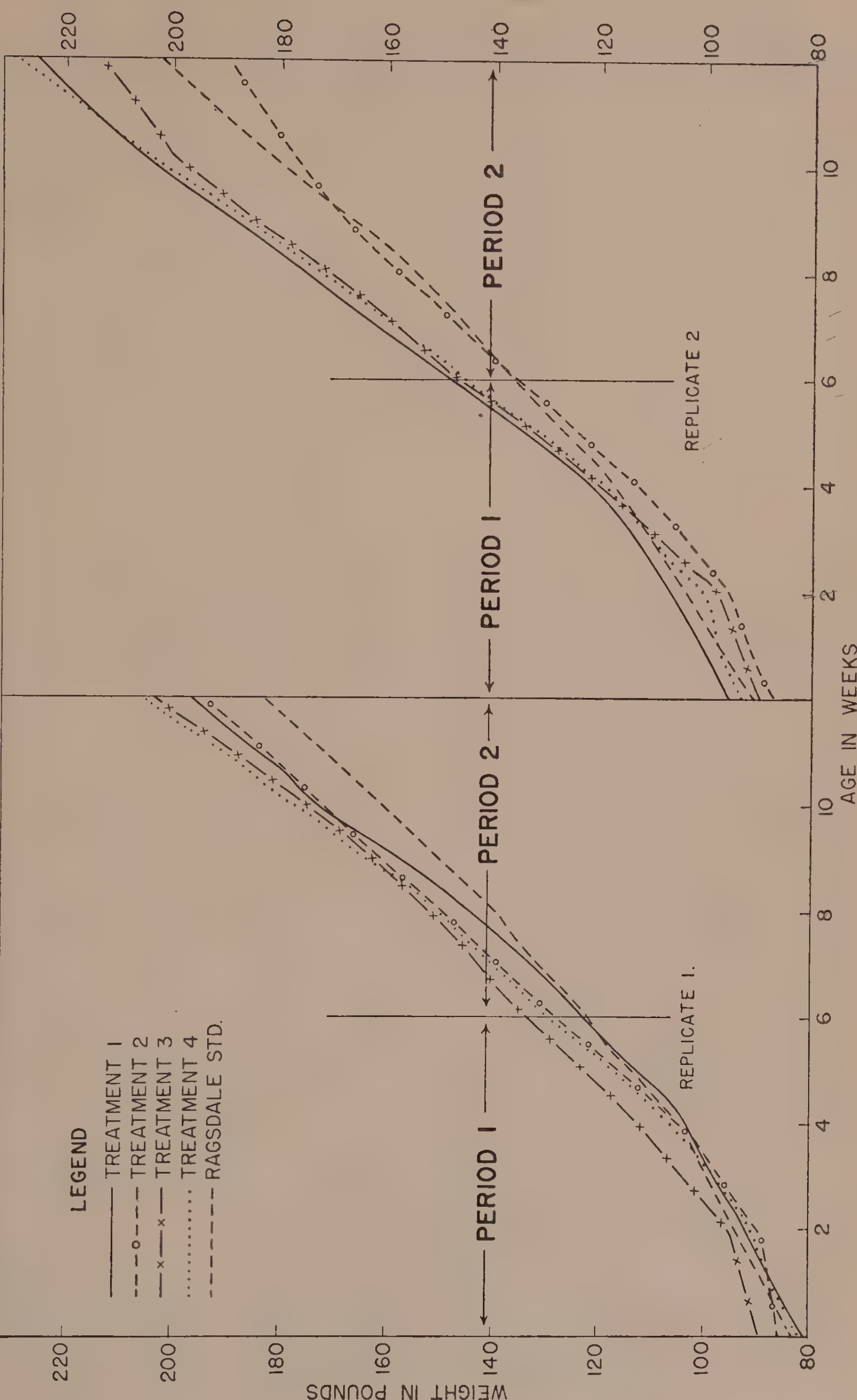


TABLE 2.—AVERAGE FEED INTAKE OF DAIRY CALVES FED AN ANTIBIOTIC FEED SUPPLEMENT (AUREOMYCIN) (COMBINED RESULTS OF REPLICATES 1 AND 2)

	I Control	II Supplement in milk for 12 wk.	III Supplement in milk first 6 wk.	IV Supplement in meal to 12 wk.
	lb.	lb.	lb.	lb.
	(Av. 6-week periods)			
Period 1				
Starter	8.9	16.4	20.9	11.2
Hay	13.1	14.4	14.1	17.5
Period 2				
Starter	135.0	120.9	125.4	141.7
Hay	106.4	77.3	98.8	107.7

response to antibiotics in chick experiments (6). It is possible that the environmental conditions such as sanitation, nutritional status, etc., under which this and other experiments (8, 15, 21) were conducted, may account for the response obtained. These results, therefore, support the belief that, under good management and feeding practices, Aureomycin may not affect the growth of young dairy calves.

Digestibility Trial

The digestibility coefficients and TDN determined in the digestibility trial are given in Table 3. The antibiotic feed supplement had no influence on the digestibility of the dry matter, ether extract or nitrogen-free extract. The crude protein and crude fibre appeared to be slightly better digested by the calves receiving the Aureomycin supplement. While this effect was noted consistently with each pair of calves, the average increments of 2.2 and 8.6 per cent respectively, for the protein and fibre coefficients, were not statistically significant. As a result of the greater digestibility of the protein and fibre, the TDN content of the ration containing antibiotic supplement was approximately 5 per cent greater than that of the control ration. This difference was not statistically significant.

No difference in the digestibility was expected, since the antibiotic supplement had no effect on growth and feed utilization in the feeding test. However, Hibbs *et al.* (9) also observed that Aureomycin feeding had no influence on the percentage of dry matter, cellulose and protein digested by calves, which under their experimental conditions exhibited a growth response to the antibiotic supplementation. It is concluded, therefore, that, under the sanitary conditions which prevailed in these experiments, low level feeding of Aureomycin had no effect on the digestion or on the body weight of young dairy calves.

TABLE 3.—COEFFICIENTS OF DIGESTIBILITY AND TDN OF DAIRY CALF RATION

	Control	Control + Antibiotic[1]
Dry matter	85	85
Organic matter	86	86
Crude protein	88	90
Crude fibre	40	49
Ether extract	89	88
Nitrogen-free extract	92	92
TDN	100	105

[1]12 mg. of Aureomycin as Aurofac A per 100 lb. body weight daily.

REFERENCES

1. Bartley, E. E., F. C. Fountaine, and F. W. Atkeson. The effects of APF concentrate containing Aureomycin on the growth and well-being of young dairy calves. J. Anim. Sci. 9 : 646-647. 1950.
2. Bartley, E. E., F. W. Atkeson, H. C. Fryer, and F. C. Fountaine. Antibiotics in dairy cattle nutrition. III. Effects of different levels of Aureomycin intake upon the growth and well-being of dairy calves and the association of differences with changes in environment. J. Dairy Sci. 37 : 259-268. 1954.
3. Bell, M. C., C. K. Whitehair, and W. D. Gallup. The effect of Aureomycin on digestion in steers. Proc. Soc. Expl. Biol. Med. 76 : 284-286. 1951.
4. Bloom, S., and C. B. Knodt. The value of Aureomycin and vitamin B_{12} in milk replacement formulas for dairy calves. J. Dairy Sci. 35 : 910-914. 1952.
5. Braude, R., S. K. Kon, and J. W. G. Porter. Antibiotics in nutrition. Nutr. Abstr. and Rev. 23 : 473-495. 1953.
6. Coates, M. E., C. D. Dickinson, G. F. Harrison, S. K. Kon, J. W. G. Porter, S. H. Cummins, and W. F. I. Cuthbertson. Mode of action of antibiotics in chick nutrition. J. Sci. Food Agr. 3 : 43-48. 1952.
7. Chance, C. M., C. K. Smith, C. F. Huffman, and C. W. Duncan. Antibiotics in rumen digestion and synthesis. III. The effect of Aureomycin on rumen micro-organisms with special reference to streptococci and coliform groups. J. Dairy Sci. 36 : 743-751. 1953.
8. Gaunya, W. S., R. D. Mochrie, R. E. Johnson, and Cecilia M. Dembiczak. Effects of adding small quantities of Aureomycin (Aurofac) to the starter of calves. J. Dairy Sci. 38 : 1-5. 1955.
9. Hibbs, J. W., H. R. Conrad, and W. D. Pounden. A high roughage system for raising calves based on the early development of rumen function. V. Some effects of feeding Aureomycin with different ratios of hay to grain. J. Dairy Sci. 37 : 724-736. 1954.
10. Knodt, C. B., E. B. Ross, and J. F. Stein. Further studies of Aureomycin in feed formula for dairy calves. J. Dairy Sci. 36 : 1201-1204. 1953.
11. Kon, S. K., J. Oliver, J. W. G. Porter, and B. Ridley. The effect of Aureomycin and Penicillin on the growth of calves. Proc. Nutr. Soc. 12 : 10. 1953.
12. Lassiter, C. A. Antibiotics as growth stimulants for dairy cattle. J. Dairy Sci. 38 : 1102-1139. 1955.
13. Logan, V. S. Antibiotics in the feeding of dairy calves. Agr. Inst. Rev. 7 : 11-13. 1952.
14. Loosli, J. K., R. H. Wasserman, and L. S. Gall. Antibiotic studies with dairy calves. J. Dairy Sci. 34 : 500. 1951.

15. Morrison, S. H., and J. F. Deal. Effect of APF (antibiotic feed supplement) supplementation during the first two weeks of life on the well-being and growth of dairy heifers. J. Anim. Sci. 10 : 1057. 1951.
16. Murley, W. R., N. L. Jacobson, and R. S. Allen. The effect of Aureomycin supplementation on growth and feed utilization of young dairy calves. J. Dairy Sci. 35 : 846-856. 1952.
17. Ragsdale, A. C. Growth standards for dairy cattle. Univ. Mo. Agr. Expt. Sta. Bull. 336. 1934.
18. Reid, J. T., K. G. Warner, J. K. Loosli. Antibiotics in the nutrition of ruminants. J. Agr. and Food Chem. 2 : 186-192. 1954.
19. Rusoff, L. L., A. V. Davis, and J. A. Alford. Growth-promoting effect of Aureomycin on young calves weaned from milk at an early age. J. Nutr. 45 : 289-300. 1951.
20. Rusoff, L. L., J. A. Alford, and C. E. Hyde. Effect of type of protein on the response of young dairy calves to Aureomycin with data on the intestinal microflora. J. Dairy Sci. 36 : 45-51. 1953.
21. Whiting, F., and R. D. Clarke. Raising dairy calves with a limited amount of milk. Can. J. Agr. Sci. 35 : 454-460. 1955.

INSECTICIDES AND VARIETAL RESISTANCE IN THE CONTROL OF THE SQUASH VINE BORER, *MELITTIA CUCURBITAE* (HARR.), IN SOUTHWESTERN ONTARIO[1]

L. A. MILLER[2]

Entomology Laboratory, Chatham, Ontario

[Received for publication May 10, 1956]

ABSTRACT

In experiments on Golden Hubbard squash in 1952 and 1954, dusts of 2½ per cent aldrin, 1 and 2½ per cent dieldrin, 3 per cent methoxychlor, and 3 per cent aerosol-grade DDT each gave excellent control of the squash vine borer. A 1 per cent rotenone dust was moderately effective. Three applications, the first during the last week of June and the two others at 7- to 10-day intervals, were adequate. The dusts should be directed to the bases of the stems, where 85 per cent of the eggs are laid. The insecticides were not phytotoxic under conditions of the experiment.

Butternut pumpkin, Honey Cream watermelon, Iroquois muskmelon, Zucca melon, and red-seeded citron and National Pickling and Straight 8 cucumbers were immune to borer attack. The following cucurbit varieties were susceptible in varying degrees: Delicata, Table Queen, Royal Acorn, Connecticut Field, and Small Sugar pumpkins; Vegetable Marrow (bush and trailing) summer squash; Mammoth Chili, Golden Delicious, Green Delicious, Blue Hubbard, Warted Hubbard, Green Hubbard, Golden Hubbard, Banana Pink, Banana Green, Buttercup and Hungarian Mammoth winter squashes; gourd.

INTRODUCTION

The squash vine borer, *Melittia cucurbitae* (Harr.), is a very serious pest of most squash and pumpkin varieties in southwestern Ontario. Infestations are frequently so severe in small market and kitchen gardens that either the vines die before setting fruit or the fruits are much inferior in quality and fewer in number. On commercial acreages, infestations rarely result in complete crop failures.

Little control is possible once the borers have entered the vines. The time-honoured method of slitting the vines lengthwise, removing the borers, and mounding over the vines with moist earth is still effective when only a few plants are infested, but is impractical when a sizable plot is involved. Similarly, crushing the eggs on the stems is 100 per cent effective in small gardens.

In Canada, Miller (6) determined that the moths emerge from the last week of June to about mid-July. Egg-laying begins the day after the female emerges, and hatching occurs approximately 9 days later. Eighty-five per cent of the eggs are laid on the basal 6 inches of the stem. For an insecticide to be effective against the borer, it must be applied at the correct time to either prevent oviposition or be lethal to the egg or first-instar larva.

Experiments on the control of the borer were conducted from 1952 to 1954. In 1953, however, the study plots were in the middle of a 5-acre field of squash and the over-all infestation was so light that significant

[1]Contribution No. 3432, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.
[2]Associate Entomologist.

results were not obtained. Heavy infestations were observed during the studies only in relatively small fields of squash or pumpkin, involving approximately a quarter of an acre.

Field observations indicated that some cucurbit varieties were less susceptible to attack than others. Consequently, in 1953 and 1954, a study was made of the relative susceptibilities of 23 varieties, including most of those popular for canning and table use as well as many of lesser importance. Howe (4, 5), in New York State, has established that the degree of infestation is attributable to resistance on the part of the host and not merely to a preference on the part of the borer. Although it was not originally planned, the related species *Cucurbita sativus* L., cucumber; *Lagenaria vulgaris* Ser., gourd; and *Lagenaria siceraria*, Zucca melon, were examined for borer infestation.

MATERIALS AND METHODS

Insecticide experiments, 1952 and 1954

In 1952 the following dust formulations were tested: $2\frac{1}{2}$ per cent aldrin, $2\frac{1}{2}$ per cent dieldrin, 3 per cent methoxychlor, and 3 per cent aerosol-grade DDT with a minimum melting point of 103°C. [Technical DDT causes severe foliage injury to most cucurbit varieties (1)]. The dusts were applied on June 25 and July 2 and 10.

In 1954 the materials tested were the same as in 1952 except that 1 per cent dieldrin was used instead of $2\frac{1}{2}$ per cent, and a specially prepared cucurbit dust containing 1 per cent rotenone and 6 per cent zineb was used as an added treatment. The dusts were applied on June 25 and July 2 and 12.

Golden Hubbard squash, a variety highly susceptible to borer attack, was used in both years.

The experimental plots were planted on June 2 and May 31 in 1952 and 1954, respectively. Each treatment was applied to 15 hills containing 5 seedlings per hill. To facilitate observations, the hills were 20 feet apart within a row and the rows were 20 feet apart. The 15 hills of each treatment were randomly distributed throughout the experimental plot. The dusts were applied with a hand-operated puff-duster. After completion of an application, the duster was flushed thoroughly with pyrophyllite before the next insecticide was used. Rates of application depended on the size of the plants and varied from 10 to 15 lb. of dust per acre for the first treatment to 30 to 40 lb. per acre for the last.

All the hills in the experimental plots were dusted with 1 per cent rotenone on June 15 in 1952, and on June 22, 1954, to control the striped cucumber beetle, *Acalymma vittata* (F.).

To determine the effectiveness of the insecticides, the following criteria were used:

(a) The total number of eggs counted on July 9 on 50 plants per treatment.

(b) The percentage infested on August 5. The presence of borers on this date was easily determined by mounds of frass along the stems and vines. In doubtful cases the vines were dissected to locate the borer.

Varietal Resistance Experiments, 1953 and 1954

Twenty-three varieties were tested for resistance to the borer. The experiment was conducted on 5 rows, each containing 23 hills. The hills, each containing 5 plants of one of the 23 varieties, were distributed at random within each row. The plots were planted on May 31 in each year. On June 15 of each year they were dusted with 1 per cent rotenone for control of the striped cucumber beetle.

The nomenclature of the varieties of cucurbit plants follows that of Graham and Shoemaker (3), who clarified much of the confusion in names for the true or winter squash, *Cucurbita maxima* Duchesne, and for the pumpkin and summer squashes, *C. pepo* L. and *C. moschata* Duchesne.

The relative resistance of the varieties to the borer was determined by counting the number of eggs on all plants of each variety on July 6, and by counting the borers in 4 vines per variety on various dates.

The varieties tested were: Mammoth Chili, Blue Hubbard, Warted Hubbard, Green Hubbard, Golden Hubbard, Green Delicious, Golden Delicious, Banana Pink, Banana Green, Buttercup, Hungarian Mammoth, and Boston Marrow, *Cucurbita maxima* Duchesne; Vegetable Marrow (bush), Vegetable Marrow (trailing), Table Queen, Royal Acorn, Connecticut Field Pumpkin, Small Sugar Pumpkin, and Delicata, *Cucurbita pepo* L.; Honey Cream Watermelon, *Citrullis vulgaris* Schrad.; Iroquois muskmelon, *Cucumis melo* L.; red-seeded citron, *Citrullis vulgaris citroides*; Butternut, *Cucurbita moschata* Duchesne.

RESULTS AND DISCUSSION

Insecticides

Table 1 shows that each of the insecticides reduced oviposition and, except for the 1 per cent rotenone dust, gave excellent control of the borer. Since hatching is continuous over approximately one month, the desirability of using insecticides with long residual actions is apparent. The

TABLE 1.—NUMBERS OF EGGS OF THE SQUASH VINE BORER ON GOLDEN HUBBARD SQUASH AND PERCENTAGES INFESTED AFTER APPLICATIONS OF VARIOUS INSECTICIDAL DUSTS, CHATHAM, ONTARIO, 1952 AND 1954

Treatment	Eggs on 50 plants on July 9		Percentage infested (50 plants) on August 5	
	1952	1954	1952	1954
Aldrin, 2½ per cent[1]	63	53	0	0
Dieldrin[2]	66	42	0	0
Methoxychlor, 3 per cent[3]	50	46	2	0
DDT, 3 per cent[4]	25	51	0	0
Rotenone, 1 per cent[5]	—	51	—	26
Check	101	114	76	82

[1]Shell Chemical Company of Canada, Toronto 1, Ont.
[2]Shell Chemical Company of Canada, Toronto 1, Ont.; 2½ per cent in 1952, 1 per cent in 1954.
[3]Cucurbit Dust, Canadian Industries Limited, Hamilton, Ont.
[4]Vinesafe, Green Cross Insecticides, Montreal 22, Que.
[5]King Cucurbit Dust (1% rotenone, 6% zineb), King Calcium Products Limited, Campbellville, Ont.

TABLE 2.—AVERAGE NUMBERS OF EGGS PER PLANT ON JULY 6 AND NUMBERS OF BORERS DISSECTED FROM 4 VINES OF EACH OF A NUMBER OF CUCURBITS ON VARIOUS DATES, CHATHAM, ONTARIO, 1953 AND 1954

Variety	Average number of eggs per plant (115 plants)		Number of borers in 4 vines							
			1953				1954			Mean*
	1953	1954	July 21	Aug. 11	Aug. 18	Sept. 11	July 22	Aug. 9	Aug. 18	1953 and 1954
Honey Cream watermelon	0	0	0	0	0	0	0	0	0	0
Iroquois muskmelon	0	0	0	0	0	0	0	0	0	0
Red-seeded citron	0	0	0	0	0	0	0	0	0	0
Butternut pumpkin	1	1	0	0	0	0	0	0	0	0
Delicata pumpkin	4	4	1	5	2	8	2	1	7	3.71
Table Queen pumpkin	4	3	0	8	9	8	5	0	6	5.14
Royal Acorn pumpkin	6	5	3	6	2	10	2	8	6	5.29
Vegetable Marrow (bush) summer squash	6	6	3	13	13	8	9	19	6	10.14
Vegetable Marrow (trailing) summer squash	3	5	1	8	15	20	4	17	17	11.71
Golden Delicious winter squash	9	5	7	23	8	6	13	13	15	12.14
Small Sugar pumpkin	5	6	7	17	8	10	8	17	20	12.42
Connecticut Field pumpkin	4	5	5	6	20	13	12	22	11	12.71
Green Hubbard winter squash	9	6	5	17	8	6	26	28	16	15.14
Green Delicious winter squash	9	9	3	26	2	9	36	20	20	16.57
Banana Pink winter squash	10	11	5	24	12	2	24	24	36	18.14
Mammoth Chili winter squash	15	11	15	21	22	10	24	25	12	18.43
Blue Hubbard winter squash	10	7	3	18	28	8	9	53	13	18.86
Golden Hubbard winter squash	9	8	3	17	17	9	32	48	11	19.57
Hungarian Mammoth winter squash	11	10	7	17	29	15	23	30	16	19.57
Banana Green winter squash	7	10	1	14	24	7	21	44	30	20.14
Boston Marrow winter squash	8	7	4	35	14	8	24	29	35	21.29
Warted Hubbard winter squash	10	8	20	17	9	7	26	49	27	22.14
Buttercup winter squash	10	7	14	26	31	15	27	30	17	22.86

*Any two means not within the same bracket are significantly different at the 5 per cent level; any two within the same bracket are not significantly different at the 5 per cent level.

comparative failure of rotenone may be attributed to its rapid deterioration on exposure to air and sunlight. None of the insecticides was phytotoxic under conditions in the plots in 1952 and 1954.

As an added observation, each of the insecticides apparently controlled the striped cucumber beetle and the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* Barber.

Resistant Varieties

Data on the relative susceptibilities of the 23 varieties to oviposition and borer establishment in the vines are summarized in Table 2. Significant differences in resistance to the borer between varieties were tested by means of the multiple range test at the 5 per cent level (2).

In addition to the varieties in the experiment showing immunity to borer establishment, cucumber and Zucca melon were also immune. Gourd was susceptible to the same degree as Small Sugar pumpkin.

ACKNOWLEDGEMENTS

Thanks are extended to Miss K. E. Boyce and E. E. Wiffen, formerly of the Chatham Laboratory, and A. J. De Lyzer of the Chatham Laboratory, who, under the direction of the author, conducted much of the work. R. J. McClanahan of the Chatham Laboratory conducted the statistical analyses.

REFERENCES

1. Carruth, L. A., and G. E. Hervey. DDT and other insecticides for squash borer control. J. Econ. Ent. 40 : 716-721. 1947.
2. Duncan, D. B. Multiple range and multiple *F* tests. Biometrics 11 : 1-7. 1955.
3. Graham, T. O., and J. S. Shoemaker. Vegetable varieties and hybrids. Ontario Dept. Agr. Bull. 451 : 77-82. 1952.
4. Howe, W. L. Factors affecting the resistance of certain cucurbits to the squash borer. J. Econ. Ent. 42 : 321-326. 1949.
5. Howe, W. L. Biology and host relationships of the squash vine borer. J. Econ. Ent. 43 : 480-483. 1950.
6. Miller, L. A. Notes on the life-history and habits of the squash vine borer, *Melittia cucurbitae* (Harr.) (Lepidoptera: Aegeriidae), in southwestern Ontario. Can. J. Agr. Sci. 35 : 533-537. 1955.

Change in Publication Policy

The publication of the *Canadian Journal of Agricultural Science* in its present form will terminate with the November-December, 1956, issue. It will be replaced by three new journals, namely:

Canadian Journal of Plant Science,

Canadian Journal of Soil Science,

Canadian Journal of Animal Science.

The *Canadian Journal of Plant Science* will be published in the months of January, April, July and October.

The *Canadian Journal of Soil Science* will be published in the months of February and August.

The *Canadian Journal of Animal Science* will be published in the months of June and December.

Publication of the new journal series will commence January, 1957, and the volume numbers for each new journal will follow the last volume of the *Canadian Journal of Agricultural Science*, i.e., each new journal to commence with Volume 37.

Subscription rates for the new journals will be as follows:

	Canada, U.S.A., Mexico, Central and South America, and British Commonwealth	*Other Countries*
Canadian Journal of Plant Science	**$4.00**	**$5.00**
Canadian Journal of Soil Science	**2.00**	**2.50**
Canadian Journal of Animal Science	**2.00**	**2.50**

The price of single numbers of all journals will be $1.25.

Present subscriptions to the *Canadian Journal of Agricultural Science* will be honoured to expiry dates.

Correspondence and manuscripts should be addressed to the appropriate journal at:

AGRICULTURAL INSTITUTE OF CANADA,
338 Somerset Street West,
Ottawa 4, Canada.